FEEDING EXPERIMENTS WITH ISOLATED FOOD-SUBSTANCES.

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Although the proteins have long had attention centered upon them because of their commanding position in relation to nutrition, it is only in very recent years that the progress of chemical investigation, fostered by the introduction of new methods of research, has begun to make our conception of these fundamentally important substances more exact. The role of the nitrogenous substances in both plants and animals has gradually been brought to light by a series of effective researches; but even to-day it is still common, despite the newer knowledge in the field of biochemistry, to read of the part played by proteins, fats, and carbohydrates in nutrition, as if these groups of compounds were each chemically homogeneous and, within wide limits, physiologically interchangeable. Not only have these food-stuffs been discussed in the past without more than the scantiest consideration of possible specific differentiations within the individual groups, but in nearly every investigation on nutrition the nutrients have been employed in those complex and often little-understood mixtures which make up the common foods.

That any such indefinite combination of known and unknown organic compounds is an unsatisfactory and unideal starting-point in any adequate study of the laws of nutrition was appreciated by Carl Voit, the foremost student of this subject of the past generation. In discussing the necessity for an accurate knowledge of the food intake in the study of metabolism he says:

> Zu dem Zwecke wäre es unstreitig am besten, könnte man nur reine, chemische Verbindungen (die reinen Nahrstoffe) z.B. reines Eiweiss, Fett, Zucker, Stärkemehl, Aschebestandtheile, oder Gemische derselben geben. Da aber die Menschen und auch die Thiere nur selten solche geschmacklose Gemenge auf die Dauer aufzunehmen oder zu ertragen vermögen, so bleibt für die meisten Fälle nichts anderes übrig als schon durch die Natur zusammengesetzte Mischungen (die Nahrungsmittel) zu wählen. Jedoch wäre es wohl möglich und ganz verdienstvoll, die Grundversuche, nachdem vorher der Weg mit Hilfe der letzteren Mischungen gefunden worden ist, mit den reinen Stoffen zu wiederholen, obwohl sich dabei sicherlich im Wesentlichen keine anderen Resultate ergeben werden.*

NOTE.—The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C. In these experiments we have been further assisted by M. S. Fine and C. S. Leavenworth.

* C. Voit: Hermann's Handbuch der Physiologie, 1881, VI, (1), p. 19.

Judging on the basis of such information as was available at the time when the preceding was written, Voit further stated in regard to the proteins:

Die verschiedene Modificationen des Eiweisses haben höchst wahrscheinlich sämmtlich ganz die gleiche Wirkung auf den Stoffumsatz, jedoch liegen hierüber noch keine genauen Untersuchungen vor.*

Certain exceptions to the assumed physiological equivalence of the proteins of various origins have long been known, conspicuously in the case of gelatin. This nitrogenous compound, possessing many of the most characteristic chemical features of a typical protein, was found to be inadequate as the sole source of nitrogen to the higher animals. Its lack of a constituent tyrosine complex and of sulphur was appreciated; and to this was often charged the insufficiency of its nutritive functions. It has remained for the newer researches on the proteins—those interesting studies of the past ten years which have unraveled so much of the structural complexity of the albuminous molecule—to raise new questions and place the problems of nutrition in a new light. It has been the structural dissimilarity rather than the likeness of the proteins which has aroused physiological comment. The rich fund which these investigations of the constituent amino-acid derivatives of the various proteins of both animal and plant origin have disclosed need not be presented here.† The more striking findings are rapidly becoming a matter of common knowledge in physiological circles. Thus it is appreciated that the zein of maize and the gliadin of wheat show distinctive features in respect to the yield of the different amino-acids obtainable from them. Zein is distinctly deficient in comparison with most other proteins: it yields no tryptophane, glycocoll, or lysine. Gliadin is characteristically rich in the glutaminic acid complex. Other equally interesting illustrations might be cited.

Are these different proteins, specific in respect to origin, biologically differentiated and individually unique in chemical make-up, of equal value for the purposes of nutrition? If they are not, how are we to explain the uniformity, apparent or real, which is exhibited in the composition of the individuals of any species living under widely divergent dietetic conditions? The flesh-eater and his herbivorous neighbor accumulate specific muscle-proteins, blood-proteins, and brain-proteins despite recognized differences in the nitrogenous intake.

The problem here presented has been both simplified and complicated by the trend of recent studies in the chemistry of digestion.

* C. Voit: Hermann's Handbuch der Physiologie, 1881, VI (1), p. 104.

† Elaborate reviews of this subject will be found in the monographs by Plimmer, Schryver, and T. B. Osborne, in the series of Monographs on Biochemistry, Longmans, Green & Co.; also Die Pflanzenproteine, Ergebnisse der Physiologie, 1910, X, pp. 47–215.

So long as it was still assumed that the proteins are only slightly modified within the alimentary tract, it was not easy to appreciate how these widely differing amino-acid complexes could be converted into a common protein or group of proteins. But with the introduction of the evidence that proteins experience a profound cleavage prior to absorption—that the organism is equipped with an outfit of enzymes easily capable of effecting such intense hydrolyses, and that the proteins in all probability normally disappear from the alimentary tract as amino-acid fragments and relatively simple polypeptides—our conception of protein assimilation has changed notably.

It is, perhaps, too early yet to determine to what extent the theories of protein metabolism and reconstruction, which Loewi and Abderhalden in particular have championed, accord with the facts of experience. Whether the organism synthesizes blood and tissue proteins from the amino-acid rests of digestion, and thus only; whether there are no rearrangements whereby one amino-acid may give rise to another; and whether under these circumstances the synthetic power of the organism is limited to a choice by the minimum of all essential protein building complexes—the so-called "Bausteine"—can not yet be profitably debated in detail.

The problem of protein synthesis is further complicated by a consideration of the activities of bacteria in relation to the food-products which enter the alimentary tract. It is now well appreciated that microorganisms grow and die in large numbers throughout the lower parts of the digestive tube, so that it is not uncommon to find the fæces to be composed in a very considerable degree of the bodies of dead bacteria. It is not unlikely that the organisms which thus perish in the intestinal canal are subject to autolytic or other digestive degradation by which their protoplasmic constituents may become available as food sources to the organism of the host. Bearing in mind the synthetic possibilities inherent in plant cells, such as bacteria represent, it is by no means beyond the bounds of reasonable interpretation to assume that the amino-acids first formed by digestion of food proteins may experience a synthesis into new forms of protein complexes prior to a subsequent digestion and utilization. Viewed in this light, the immediate hydrolysis products of our foodstuffs may become available only after they have been in greater or less part reconstructed by these preeminently synthetic symbiotic bacteria into products of more uniform character, possibly widely different from the original intake. Nucleoprotein synthesis, for example, may thus become referable to bacterial intervention.

No one can say at the present time to what extent, if at all, such synthetic possibilities enter into the problems here discussed. The subject has recently again been referred to by Lüthje.* The

*Lüthje: Ergebnisse der Physiologie, 1908, VII, p. 826.

important point for us is that we must deal with such possibilities; we must plan and interpret our nutrition experiments to-day in the light of these newer ideas.

If we accept the synthetic theory of metabolism, disregarding the cooperation of bacteria, it becomes possible to understand how the lack of some fundamental unit, like tyrosine, tryptophane, lysine, or cystine may lead to malnutrition or death through the inability of the organism to construct normal protein and normal protoplasm, because of a deficit of essential structural complexes. The errors of a one-sided diet present a new point of view. The importance of these theories is enormous, not alone for the nutrition of man but also for the welfare of our domestic animals. Rational and economical feeding is based upon a correct interpretation.

Considerations such as the foregoing certainly justify an extension of experimental work in nutrition along the lines suggested by the rapidly accumulating data on the structure of the individual proteins. The theories must be subjected to the rigorous test of experiment. The relative and absolute value of individual proteins must be determined by physiological trials as a preliminary to definite and more permanent, rational dietary programs. We have attempted the beginnings of such a study.

At the outset we were confronted by the fact that there are on record few, if any, successful experiments in feeding isolated foodstuffs. Still fewer are those in which the protein compound was fed isolated, in a reasonable degree of purity. By "successful" we mean experiments in which the health and vigor of animals were maintained under adequately controlled conditions for sufficient periods of time to permit of more than tentative conclusions. The total number of feeding trials in which the role of the protein food is in some way concerned is very large. We shall omit reference to all except those which bear specifically on the problems under consideration, viz., the significance of individual proteins in nutrition.

It may not be amiss at the outset to point out the broader requirements which any adequate dietetic régime involves and upon which its nutritive success depends in good measure. In the first place the nutrients must be presented in a form that is digestible and thus available for physiological utilization. The physical texture as well as digestibility *per se* plays an important part in this respect. Again, the available parts of the diet must be adequate in amount to cover the calorific needs of the organism to which it is supplied, *i.e.*, there must be sufficient metabolizable energy. One might be inclined to omit reference to such apparently obvious facts had they not been serious factors in previous experimental failures in feeding isolated food-substances. In recent years much emphasis has further been laid upon certain less evident considerations involving nutrition more

indirectly. Thus the question of the inorganic constituents of the diet is far from settled, and this aspect of dietetics can scarcely be said to rest upon any very rational basis. We realize the need of chlorine and phosphorus, of calcium and iron in certain vital functions; but the physiological requirement of other elements is none the less definite because it is not fully understood. On one point, namely, the power of the animal organism to employ the elements phosphorus and iron in the form of either organic or inorganic compounds, we have assumed that the consensus of opinion and the preponderance of experimental evidence are in favor of either possibility. The debate on this topic need not be reviewed here.

Closely related to these nutritive requirements is the subject of those food accessories which determine in large measure what is spoken of as the palatability of any ration. They act in manifold indirect ways to influence the digestion of the nutrients by their effect in promoting or retarding secretion into the alimentary tract; they affect the appetite and the psychic element in digestion—all of which have received attention anew through Pawlow's efforts. After all one is as yet not justified in insisting that these incidental features of the dietary are absolutely indispensable. Quoting Tangl:

> Eine fördernde Wirkung der sog. Reiz—oder Würzstoffe—sagt Kellner—auf die Ausnutzung des Futters ist bis jetzt bei keiner einzigen der auf diesen Punkt gerichteten Untersuchungen beobachtet worden. Dasselbe gilt nicht nur für die landwirtschaftlichen Nutztiere, sondern auch für den Menschen.*

The undoubted biological importance of that rather vaguely defined and hetereogeneous group of compounds known as lipoids has raised the question as to whether they are at all necessary in any complete diet, or whether they can be synthesized by animals as they are by plants. The lipoids (phosphatides and cholesterols) are present as common cell derivatives in every familiar food and can only be excluded by special, laborious methods of extraction. It is not strange, therefore, that there is a paucity of evidence relating to their absolute significance as constituents of the food-intake. Recent experiments by Stepp† on the indispensability of the ether-soluble constituents of the food for the life of mice are far from conclusive.

Lastly, it seems worth while to point out that the nutritive conditions which pertain during the period of growth are in many respects—perhaps far more than is realized—different from those existing at a later period. During the active constructive phase new material must be supplied which differs both in quality and amount from the quota furnished as a maintenance ration. We realize well enough that the growing skeleton requires calcium; but to what

*Tangl: Oppenheimer's Handbuch der Biochemie, 1910, III (2), p. 55.
†Stepp: Biochemische Zeitschrift, 1909, XXII, p. 452.

degree are the further nutritive demands modified by developmental changes? Are the same proteins which suffice the adult capable of supplying the essential building-stones to the young? Here again a wealth of new problems still confronts us.

There are no recorded experiments on the larger animals, such as are commonly used in laboratory work, which furnish any conclusive data on the broader questions involved in this research. The careful experiments of Röhmann and his pupils* on dogs fed with isolated proteins (edestin, vitellin, casein, myosin) extended over 50 days as a maximum period in one case. Such investigations, as well as those of Abderhalden and his collaborators,† have yielded much of interest in regard to the utilization of the food-stuffs investigated and have given valuable hints for future work in nutrition. But the periods of observation have been far too brief to permit any permanent positive conclusions regarding the adequacy of the proteins fed. Minor deficiencies may fail to become conspicuous in even comparatively long periods in the case of animals whose size and span of life indicate a considerable store of reserve material.

For various reasons the most successful investigations in this field have been conducted on much smaller animals, especially rats and mice. The preparation of suitable pure food supplies on a scale sufficient for long periods and in economically procurable amounts is thereby rendered possible. The necessary scientific measurements and analyses can be conducted on a scale impossible for larger animals, and the problem of care and attention is equally simplified. Experiments can be duplicated without great effort and individual peculiarities eliminated by force of numbers. Furthermore, the various stages of growth and maturity are completed in the smaller animals within relatively short periods of time, so that the permanent effects of any dietary become apparent within a range of days or months that is not outside of ordinary experimental possibilities of observation. As illustrations of some of these features it may be recalled that the ultimate effects of complete inanition are apparent in four or five days in rats or mice; in dogs the fatal outcome may be delayed for many weeks. The duration of life in the white rat is about three years; about 280 days suffice to complete the entire period of growth to maturity.

To what extent, if any, the rigorous conditions, such as restraint in cages, etc., set by laboratory requirements may modify the normal physiological functions of the small animals is not apparent from such records as we have found. It should be noted that both rats

*Marcuse: Archiv f. d. ges. Physiologie 1896, LXIV; 1897, LXVII, p. 373.
Steinitz: *Ibid*, 1898, LXXII, p. 75.
R. Leipziger: Inaugural Dissertation, Breslau, 1899.
E. Ehrlich: Inaugural Dissertation, Breslau, 1900.

†Cf. papers by Abderhalden and collaborators in numerous recent volumes of the Zeitschrift f. physiol. Chemie.

and mice are reported to have been kept in health on milk alone during periods of six months or more.* So far as we are aware no experiments in which "artificial" food mixtures were introduced have ever been carried on successfully for an equal duration.

The most elaborate published nutrition experiments in respect to the variety of important details taken into consideration are those of Henriques† on white rats. He determined the changes in weight and the food-intake, as well as the complete daily nitrogen balance, in a large number of animals fed on artificially mixed diets. The isolated proteins used were casein, zein, and gliadin, with additions of sugar, lard, cellulose, and inorganic salts.‡ Only with casein and gliadin were the nitrogen balances favorable for the few days during which each trial was continued. This was true despite the gradual loss of body-weight noted in each case. It serves to emphasize what is frequently overlooked, viz., that a favorable nitrogen balance over a short period of time is in no sense an adequate index to a satisfactory nutritive condition. We have found that the body-weight of the rat is a more reliable guide to the real nutritive equilibrium of the animals than is the nitrogen balance.||

Valuable as the experiments of Henriques and Hansen have been for the purposes of orientation in a new field of research, the failures can not be adduced as proof of the inadequacy of the proteins selected, nor can the apparently successful results be accepted as conclusive. For the latter point, the duration of the experiments is far too short, as will be seen from the work of subsequent experimenters. The most serious criticism, perhaps, pertains to the food intake, which must have been within the lower limits of a *maintenance* ration for the animals which in many cases had not yet completed their growth. In respect to one conclusion the words of Henriques may be quoted:

> Wir finden also, dass es eine Wesensverschiedenheit zwischen der Bedeutung des Zeins und der des Gliadins für den Stickstoffumsatz im Körper gibt; das Zein vermag kein Stickstoffgleichgewicht herzustellen, was dagegen das Gliadin vermag wenn es in reichlicher Menge gegeben wird. Der Grund dieser Verschiedenheit lässt sich natürlich nicht mit

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*Falta and Noeggerath: Hofmeister's Beiträge zur chemischen Physiologie, 1905, VII, p. 320 (rats). Knapp: Zeitschrift für experimentelle Pathologie, 1908, V, p. 150, says: "Bekanntlich können z.B. Mäuse mit Milch monatelang ernährt werden und befinden sich wohl dabei." Socin: Zeitschrift für physiologische Chemie, 1891, XV, p. 93, fed mice 99 days on egg yolk alone.

†Henriques and Hansen: Zeitschrift für physiologische Chemie, 1904–5, XLIII, p. 417; 1908, LIV, p. 169; Henriques: *Ibid.*, 1909, LX, p. 105.

‡It should be noted that these included "Knochenmehl" (p. 419–420), which is not entirely free from nitrogenous matter. Was bone-ash intended?

||It may be worth while to point out here that we have found very considerable losses of nitrogen, especially in hot weather, when urine is collected by the method adopted by Henriques and by us, which will be described later. Such losses, amounting to 10 per cent and over, would obviously lead to the assumption of a greater retention of nitrogen than actually occurred and a consequent incorrect nitrogen balance.

Sicherheit angeben. Wir haben bereits angeführt, dass es beiden genannten Proteinen an Lysin abgeht. Dieser Mangel scheint also nicht von entschiedener Bedeutung zu sein. Dagegen scheint der Umstand, dass das Zein kein Tryptophan enthalt, eine grosse Rolle zu spielen. Ob das Nichtvorhandensein von Tryptophan, in Zein wirklich der Grund ist, weshalb bei Fütterung dieses Proteins kein Stickstoffgleichgewicht eintritt, muss sich übrigens durch eine Untersuchung entscheiden lassen, ob das Stickstoffgleichgewicht sich herstellen lässt, wenn das Futter Zein+Tryptophan enthält.*

The preceding quotation has suggested that the deficiency in a protein dietary need not be one of quantity; the structural character of the nitrogenous intake may determine its adequacy. Willcock and Hopkins† have approached the problem from this view-point. They fed mice on zein together with non-nitrogenous foods and compared their length of life with that of mice which received in addition some of the missing fragments of this "imperfect" protein, viz., tryptophane (and tyrosine for comparison). Zein was shown to be quite unable to take the place of a normal protein, like casein, in maintaining growth:

> The addition of the missing tryptophane group has no power to convert such loss (of weight) into equilibrium or gain—a fact possibly due to other deficiencies in the zein molecule, such as the absence of lysine. On the other hand, on the average, the loss of weight was slower with tryptophane than without it. But this result might well be expected, even if the tryptophane administered undergoes utilization without directly contributing to tissue formation or structural maintenance. If it serves as a basis for the elaboration of a substance absolutely necessary to life—something, for instance, of an importance equal to that of adrenaline—then, in starvation, or when it is absent from the diet, a supply is likely to be maintained from the tissue-proteids; the demand for it would become one of the factors determining tissue breakdown. In the case of young animals which directly benefit from the addition of a protein constituent, otherwise absent from their diet, to the extent of a well-nigh doubled life and marked improvement in general condition, but at the same time steadily lose, instead of gaining, weight, the utilization of the constituent would appear to be of some direct and specific nature. (p. 101.)

The suggestion of a possibility of the direct formation of essential hormones from amino-acid derivatives of proteins is timely. One can not draw any further conclusions regarding the value of the proteins (zein and casein) fed by Willcock and Hopkins, because in the absence of definite intake records, the question of a comparable and adequate supply of energy in the various cases remains undetermined. Special experiments showed that the prepared zein was "in no sense actively deleterious."

The same uncertainty regarding the real participation of inanition factors applies to the earlier widely quoted experiments of

*Henriques: Zeitschrift für physiologische Chemie, 1909, LX, p. 117.
†Willcock and Hopkins: Journal of Physiology, 1906–7, XXXV, p. 88.

Lunin* on mice. Some of the animals were fed on casein as the sole protein. They survived only a month or less on the "artificial" diet, the author attributing the decline to defects in the intake of inorganic salts and other accessory foods.

Abderhalden and Rona† fed mice with casein, sugar, and sodium carbonate. The animals lived only 8 to 24 days on this obviously inadequate diet.

Falta and Noeggerath‡ recorded the changes of weight in rats kept on dietaries containing the nitrogen in the form of isolated (commercial) proteins: ovalbumin, casein, serum albumin, serum globulin, fibrin, and hæmoglobin. They fed no "roughage" in the form of cellulose. Neither these individual proteins nor mixtures of all were adequate to keep the animals alive longer than 94 days in the most favorable case. In most cases a gradual, steady decline was noted throughout the progress of the experiment. Death occurred when the animals had been reduced to two-thirds or three-fifths of their initial weight. The experiments clearly demonstrate the necessity of *prolonged* observation; for rats were maintained on the casein food mixture during several weeks without loss of weight, just as in Henriques' briefer trials of three to four weeks. Only later did the untoward effects manifest themselves. On milk, milk powder, or lean horse-meat rats could be kept six months or more.

The authors are properly guarded in their summary. They say:

> Ob in der Periode des Gewichtabfalles in unseren Versuchen die Tiere genügend Nahrung aufgenommen haben, um ihr Kalorienbedürfnis zu decken, können wir nicht sicher angeben. Der Befund von Nahrungsresten im Digestionstractus der toten Tiere (Lunin) ist unseres Erachtens hierfür kein zwingender Beweis. Hier müssten genaue Stoffwechselversuche mit Berücksichtigung der Kraftbilanz einsetzen. Erst wenn der Einwand ungenügender Nahrungsaufnahme oder ungenügender Ausnützung, für welche beiden Momente man vielleicht die Einförmigkeit der Kost und den Mangel an Gewürzen verantwortlich machen könnte, beseitigt ist wären andere Gründe zu erörtern, wie z.B. der Mangel der nötigen chemischen Bausteine oder ein abweichendes chemisches Gefüge der eingeführten Nährstoffe. (p. 322.)

Utilizing the experience gained in the preceding research, Knapp‖ succeeded no better in maintaining his animals. He estimates the calorific needs of a 200 to 250 gram rat at 50 to 60 calories. The curves of change of body-weight and caloric intake go more or less parallel in Knapp's experiments and he notes that the specific role of individual nutrients can not be suitably ascertained until animals can be induced in some way to eat the requisite amount of calories

*Lunin: Zeitschrift für physiologische Chemie, 1881, v, p. 31.

†Abderhalden and Rona: Zeitschrift für physiologische Chemie, 1904, XLII, p. 528.

‡Falta and Noeggerath: Hofmeister's Beiträge zur chemischen Physiologie, 1905, VII, p. 314.

‖Knapp: Zeitschrift für experimentelle Pathologie, 1908, v, p. 147.

in the form of the "artificial" mixtures. The danger of drawing erroneous conclusions is attested by the fact that he was unable to maintain his rats upon a variety of food articles: dog biscuit, graham bread, rice, etc., which one would assume to be adequate as mixed food.

In explanation of his failures he says:

> Der Grund liegt hauptsächlich darin, dass die Thiere bei der reizlosen einförmigen Kost den Appetit verlieren, im geringeren Grade wohl auch darin, dass die Nahrung mit zunehmender Appetitlosigkeit im Darm auch weniger gut ausgenützt wird. (p. 168.)

It might have been expected that the difficulties here encountered could be obviated by the use of animals in which forced feeding could be satisfactorily instituted. C. Voit* kept a pigeon alive 124 days with peas by this method. Jacob† failed in repeated trials with pure food mixtures, and attributes his lack of success to the impossibility of adapting the physical texture of the introduced pure-food pellets to the requirements of the avian digestion apparatus, so that the pigeons died of inanition. He also attempted to advance beyond his predecessors in feeding "artificial" mixtures (with casein as the sole protein) to rats. He was scarcely more successful; the animals ate sparingly and he concludes that if it is possible to keep an animal alive 124 days, as he did, on a diet of the character noted, this must contain all the substances essential to life. The animals did not exhibit any gross pathological defects at autopsy, but all visible fat had disappeared. The next attempt, Jacob optimistically suggests, must be directed toward devising combinations which the animals will eat:

> So gelingt es vielleicht doch, eine Nahrung aus reinen Nahrstoffen ohne Genussmittel herzustellen, welche alle zur dauernden Erhaltung eines Tieres nötigen Stoffe im richtigen Mengenverhältnis enthält. (p. 60.)

McCollum‡ fed both young and full-grown rats on complex artificial mixtures, in which edestin, zein, and sometimes casein, were the sole sources of nitrogen. They are the most successful experiments yet reported as regards maintenance of body-weight or growth on a restricted quality of protein intake. The chief difficulty encountered was that of anorexia, which the author attempted to overcome by frequent changes in the combinations of food-stuffs used and by addition of flavors. Some of the trials extended over more than 100 days without death; but the rats failed to maintain their weights, even with the most persistent coaxing of the appetite. Data regarding the food intake are wanting, so that the inanition factor (due to deficient calories) can not be excluded.

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*Voit: Zeitschrift für Biologie, 1866, II, p. 64.
†Jacob: Zeitschrift für Biologie, 1906, XLVIII, p. 19.
‡McCollum: American Journal of Physiology, 1909, XXV, p. 120.

More interesting even are his studies on the growth of young rats. They made considerable gains in weight in experiments with the proteins mentioned—without casein in one series and with it in the other—and extending over from 56 to 127 days. But the curves of growth do not approach those obtained for normal diet by Donaldson, to which we shall have occasion to refer again. McCollum concludes that "the palatability of the ration is the most important factor in animal nutrition" and "the failure of previous efforts to maintain animals on a mixture of relatively pure proximate constituents of our food-stuffs was due to the lack of palatability of such mixtures."

Finally reference must be made to the very successful attempts of Röhmann.* The details have not yet been published. Mice could be kept indefinitely on a mixture of casein, vitellin, egg albumin, and non-nitrogenous nutrients. They became mature and produced young which thrived. With only a single protein in the ration, a decline soon set in. If casein and egg albumin were used to replace each other the grown mice retained their weight, but the development of the younger ones was checked and they succumbed. Röhmann concluded from these facts that the different proteins possess a different significance for the nutrition and the development of young organisms.

Although none of our predecessors has returned a decisive answer to the fundamental question whether any single protein or combination of proteins is incapable of supplying all the essential chemical complexes which the body is unable to furnish to itself by direct synthesis, the pursuit of a solution by no means appears futile. None of the difficulties—actual or assumed—which have arisen appears experimentally insurmountable at present. The digestibility and utilization of the artificial rations has never been demonstrated to be abnormal or even unfavorable. The texture and inclusion of "roughage," such as is ordinarily a part of every mixed dietary in the guise of cellulose, can be experimentally adjusted, if indeed it is of any serious moment in controlling the mechanical functions of the alimentary tract.

Monotony of diet appears to have been overemphasized, if one may judge by the success with which milk or egg yolk have constituted the only food material for rats and mice. The failure common to all of the recorded experiments has been attributed to the difficulty of inducing animals to *eat sufficient food*. Strictly speaking, it has not been determined as yet whether the notable anorexia is the result of some unpalatable feature of the artificial food mixtures and thus the cause of the gradual inanition, or whether it is really a physiological sequence of an imperfect dietary.

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*Röhmann: Allgemeine Med. Central-Zeitung, 1903, No. 1; 1908, No. 9. Cf. Maly's Jahresbericht, 1903, XXXIII, p. 823; 1908, XXXVIII, p. 659, for the same abstracts.

The best hope of success—if such be possible—rests at present on the method of trial and error in which each variable is gradually eliminated by successive comparative experiments. This is the scheme which we have in large measure pursued. Our efforts have at first been directed toward devising a simple ration of isolated food components which should satisfy the numerous requirements set more adequately than any yet proposed. This established, substitutions could gradually be instituted in respect to the protein constituents. We learned before long that a diet which might be adequate for maintenance was by no means necessarily suited to the requirement of a growing animal. Hence our attention has become directed to some of the features of growth as well as those of the maintenance ration of the adult.

METHODS EMPLOYED.

The rats were kept in metabolism cages similar to that described by Henriques and Hansen.* A small door permits the introduction of food and water cups, such as are used in bird cages, through the side of the cage.

Figure A shows the essential features. Instead of weighing the food (which was always fed in the form of a homogeneous paste) in the food cups, we devised the following very simple plan to avoid frequent weighings. The food is introduced into a glass cylinder about 25 cm. in length and 3 cm. in diameter. A rubber stopper inserted into one end can be moved forward like a piston head and the food expelled from the other end of the cylinder into the food receptacle. The exit end of the cylinder is kept stopped when the food is not being expelled and the entire apparatus with its food content can be preserved in an ice-box for long periods without deterioration of the diet. The food eaten can thus be renewed at intervals and the quantity fed determined, when desired, by ascertaining the loss of weight of each food tube.

Figure B illustrates our feeding-tube device.

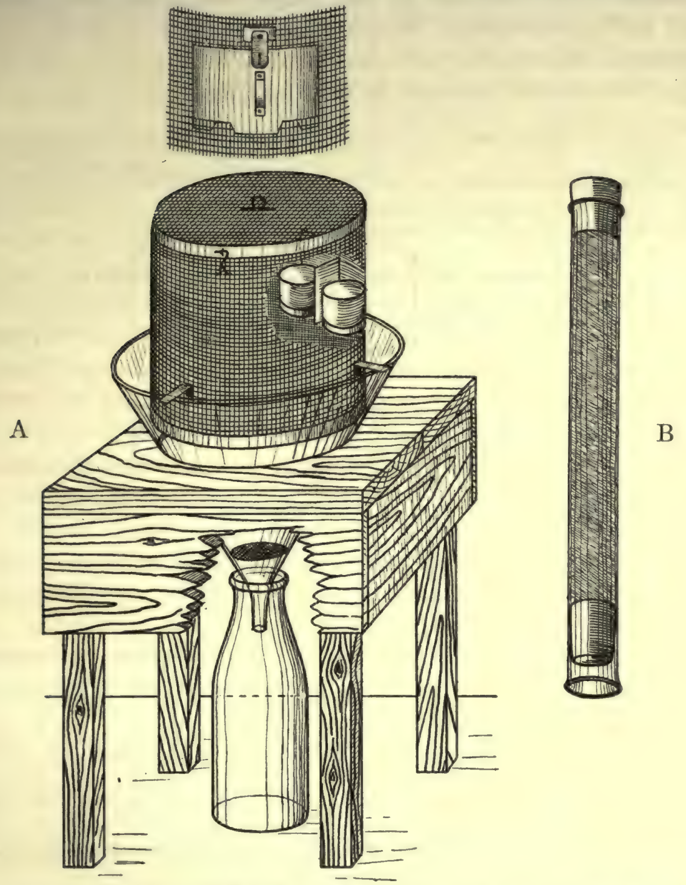
The urine and fæces were separately collected, the former in a receptacle containing boric acid and chloroform, and analyzed at intervals as indicated in the protocols. Control trials made by trickling known volumes of analyzed human urine over the cage bottom, and, after a suitable interval, washing with boric acid solution, collecting the urine, etc., just as in the rat experiments, indicated losses of 10 per cent or more. This must be borne in mind in considering our results and presumably those of other investigators.

We devoted great care to maintaining suitable environmental conditions (temperature, etc.), since the rats are sensitive to marked changes. With our diets they consumed large quantities of water.

*Henriques and Hansen: Zeitschrift für physiologische Chemie, 1904–5, XLIII, p. 418.

The success of the cage methods, as such, is shown by our ability to maintain animals in good health for very long periods in this way.

Confronted at the outset with the necessity of ascertaining whether the conditions selected—the caging, laboratory environment, consistency of the food and the mode of feeding, etc.—were



A. Sketch of cage used for feeding and collection of urine and fæces. Upper figure shows outer view of food-and-water-receptacle. (Reduced to one-twelfth natural size.)
B. Illustration of tube from which daily ration is discharged during each diet period. (Reduced to one-fourth natural size.)

endurable for the animals under any system of feeding, we undertook control trials with a mixed food in the form of dog biscuit and lard. This was prepared as follows:

The dry dog biscuits were ground to a moderately fine powder in a mill and usually 70 parts by weight were mixed with 30 parts of melted lard. The mixture when cooled was reduced to a homogeneous paste by passing several times through a meat-chopping machine. In this way the paste was forced through small holes in numerous filaments, to which a rotary motion was imparted, insuring a very

complete and rapid mixing of the ingredients of the paste. This method of mixing was used for all of the foods described in this paper.

A series of rats was fed with this food at the same time that other experimental diets were being investigated. In this way all the animals were exposed to the same indeterminable variables of climate and environment which might perchance have exerted an unsuspected deleterious influence and which would exhibit themselves in the control animals as well as those under special observa-

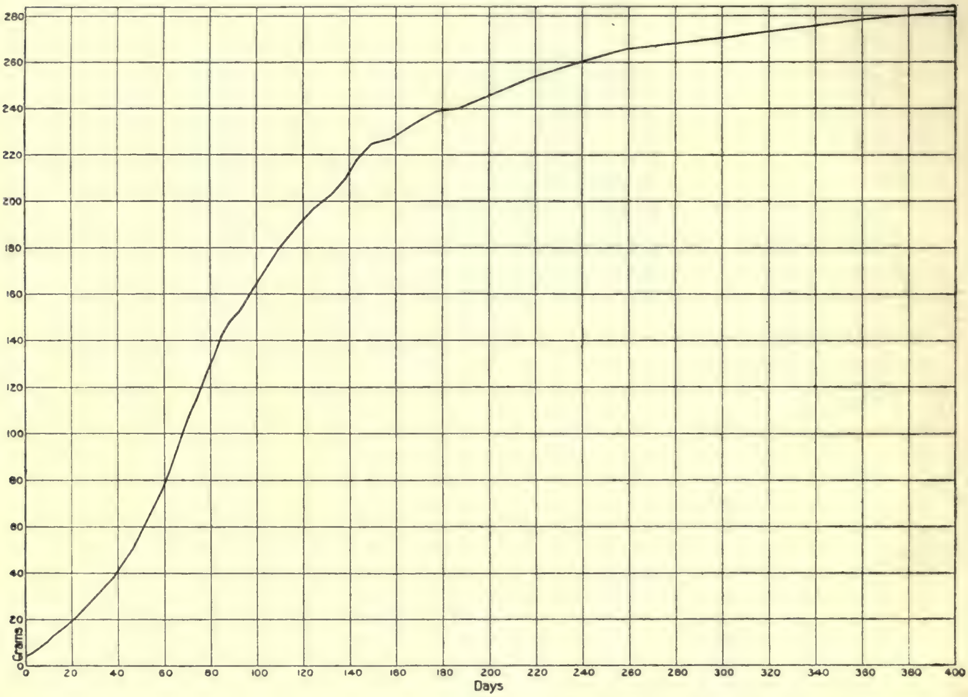


CHART I.—Average Normal Rate of Growth of Male White Rat, according to Donaldson.

tion. Like most of our trials during the first year of this work, they served chiefly for the purpose of orientation in respect to future procedure. It is scarcely necessary to record here the numerous individual experiments which resulted in a failure to maintain the rats in health and nutritive equilibrium. Failures, unless they are invariable in their occurrence, may well be due to accidents or incidents in no wise associated directly with the nutritive functions. Intercurrent parasitic diseases, incipient senility, hereditary defects, and other incidental features may be present or arise to interfere with the normal progress of an experiment. We have gradually learned to watch for such undesirable conditions and to exclude such animals as unsuitable for these studies, since proper allowance can

not be made for the perversions of function thereby introduced. For this reason we have been inclined to lay stress upon only those experiments which were either successful or which failed because of obvious causes.

In selecting criteria of adequate growth the painstaking statistical studies of Donaldson* on the adjustment of size to body-weight and age in the white rat have been of great help. After birth the young white rat depends upon the mother for sustenance for about 20 days. The span of life is about three years. Sexual maturity is reached in about 60 days. The first year of rat life corresponds, according to Donaldson, to the first thirty years of human life; and the growth curve for this period has been published by him. Some of the details are reproduced in Chart I.

The lack of appreciation of the salient features of these curves, representing graphically the gross normal increase in weight of white rats during the first third of their life, has led occasionally to conclusions which appear to us as quite erroneous. If, for example, a rat weighing 250 grams maintains its body-weight for several weeks without marked variations one may properly conclude that a normal nutritive equilibrium exists in such an animal; on the other hand a rat whose initial weight is 70 grams is in a period of most active growth. *Normal* nutrition for an animal in *this* phase calls for a measurable daily increment in weight and a gradual, yet detectable, increase in body-length. Within one month a 70-gram white rat ordinarily will double its weight when the diet is adequate. The illustrations cited suffice to indicate how different must be our criteria for the adolescent and the adult stages.

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*Donaldson: A comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume, 1906.

EXPERIMENTAL PART.

CONTROL FEEDING.

A somewhat detailed protocol of the metabolism experiments on two of the "control" rats fed on dog biscuit and lard, as already mentioned, will serve as a typical description of the conduct of all our feeding trials. Rat XII and rat XIII were caged separately on August 9, 1909. Fresh food-paste (see page 13) was introduced daily into the food dishes in excess of the amount eaten, which was at first ascertained daily. The body-weights were at first determined every other day, as was the nitrogen of the excreta (urine and fæces). Subsequently it was found adequate to estimate the nitrogen balance in weekly periods. The data thus obtained are summarized

TABLE I.—SUMMARY OF DATA ON "CONTROL" RAT XII, FED ON DOG BISCUIT-LARD DIET FOR 147 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Aug. 8....	143.5							
10....	150.0	9.5	0.143	0.107	0.027	0.134	81	+0.009
12....	155.0	9.2	0.139	0.103	0.029	0.132	79	+0.007
14....	157.7	8.7	0.132	0.096	0.037	0.133	72	−0.001
16....	156.5	7.8	0.124	0.098	0.030	0.128	76	−0.004
18....	162.0	9.3	0.149	0.106	0.012	0.118	92	+0.031
20....	165.5	9.1	0.144	0.106	0.031	0.137	78	+0.007
22....	165.0	8.1	0.129	0.088	0.021	0.109	84	+0.020
24....	164.1	7.0	0.112	0.080	0.022	0.102	80	+0.010
26....	165.5	6.6	0.103	0.075	0.022	0.097	79	+0.006
28....	165.2	6.7	0.105	0.073	0.021	0.094	80	+0.011
30....	162.6	5.8	0.091	0.069	0.027	0.096	70	−0.005
Sept. 1....	160.0	6.2	0.097	0.067	0.024	0.091	75	+0.006
3....	159.6	6.2	0.096	0.064	0.015	0.079	84	+0.017
5....	159.7	7.3	0.113	0.073	0.025	0.098	78	+0.015
7....	161.4	8.1	0.133	0.076	0.038	0.114	71	+0.019
9....	158.6	6.2	0.102	0.079	0.027	0.106	74	−0.004
11....	160.7	7.5	0.123	0.063	0.027	0.090	78	+0.033
13....	157.2	6.2	0.101	0.063	0.024	0.087	76	+0.014
15....	157.9	6.1	0.099	0.061	0.023	0.084	77	+0.015
17....	157.6	6.6	0.107	0.066	0.029	0.095	73	+0.012
19....	159.5	7.5	0.123	0.073	0.028	0.101	77	+0.022
26....	157.8	6.7	0.111	0.065	0.030	0.095	73	+0.016
Oct. 3....	154.8	5.5	0.091	0.080	0.022	0.102	76	−0.011
11....	158.5	6.4	0.094	0.067	0.018	0.085	81	+0.009
17....	150.0	4.5	0.074	0.082	0.023	0.105	69	−0.031
24....	147.5	5.1	0.084	0.067	0.015	0.082	82	+0.002
31....	142.2	4.8	0.080	0.052	0.020	0.072	75	+0.008
Nov. 7....	148.0	5.4	0.090	0.055	0.016	0.071	82	+0.019
14....	141.1	3.9	0.065	0.050	0.014	0.064	78	+0.001
21....	142.7	4.6	0.077	0.057	0.015	0.072	81	+0.005
28....	143.7	4.7	0.079	0.049	0.020	0.069	75	+0.010
Dec. 5....	141.3	4.3	0.071	0.057	0.015	0.072	79	−0.001
12....	147.3	5.0	0.083	0.058	0.014	0.072	83	+0.011
19....	152.2	5.4	0.090	0.058	0.015	0.073	83	+0.017
26....	150.4	5.1	0.084	0.063	0.016	0.079	81	+0.005
1910.								
Jan. 2....	151.7	4.8	0.079	0.057	0.017	0.074	78	+0.005

in tables and also reproduced in graphic form. This experiment in common with many others was concluded after 153 days on January 10, 1910, by a fire which destroyed all of our experimental animals.

Tables I and II give the data for the "control" rats XII and XIII, which had been obtained up to January 3, 1910, a period of 147 days, at the end of which time both rats showed a distinct gain in weight and a considerable gain of nitrogen:

TABLE II.—SUMMARY OF DATA ON "CONTROL" RAT XIII, FED ON DOG BISCUIT-LARD DIET FOR 147 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Aug. 8....	255.5	......	......	......	......	......	......	......
10....	266.0	12.4	0.187	0.145	0.039	0.184	79	+0.003
12....	268.8	11.1	0.168	0.197	0.041	0.238	76	−0.070
14....	275.7	12.7	0.191	0.126	0.038	0.164	80	+0.027
16....	280.0	12.1	0.192	0.171	0.039	0.210	80	−0.018
18....	287.5	13.4	0.214	0.133	0.044	0.177	79	+0.037
20....	296.1	13.2	0.210	0.177	0.041	0.218	80	−0.008
22....	303.5	13.6	0.216	0.146	0.038	0.184	82	+0.032
24....	307.0	13.8	0.219	0.150	0.047	0.197	79	+0.022
26....	317.0	13.6	0.213	0.149	0.044	0.193	79	+0.020
28....	325.0	13.8	0.216	0.152	0.042	0.194	81	+0.022
30....	329.5	13.7	0.214	0.144	0.040	0.184	81	+0.030
Sept. 1....	333.1	13.6	0.212	0.141	0.046	0.187	78	+0.025
3....	328.2	12.3	0.192	0.134	0.034	0.168	82	+0.024
5....	327.0	13.3	0.207	0.139	0.043	0.182	79	+0.025
7....	327.2	13.0	0.212	0.141	0.043	0.184	80	+0.028
9....	325.8	12.5	0.203	0.146	0.040	0.186	80	+0.017
11....	329.0	11.9	0.194	0.129	0.036	0.165	81	+0.029
13....	328.1	11.9	0.194	0.140	0.036	0.176	81	+0.018
15....	330.6	12.7	0.207	0.138	0.038	0.176	82	+0.031
17....	329.8	12.4	0.202	0.140	0.038	0.178	81	+0.024
19....	331.9	12.1	0.197	0.147	0.030	0.177	85	+0.020
26....	319.7	11.2	0.187	0.127	0.038	0.165	80	+0.022
Oct. 3....	322.8	12.5	0.207	0.159	0.034	0.193	84	+0.014
11....	334.7	13.9	0.198	0.137	0.038	0.175	81	+0.023
17....	335.8	12.2	0.200	0.171	0.042	0.213	79	−0.013
24....	334.5	10.5	0.175	0.126	0.031	0.157	82	+0.018
31....	330.5	10.3	0.171	0.142	0.030	0.172	82	−0.001
Nov. 7....	332.0	10.9	0.183	0.131	0.033	0.164	82	+0.019
14....	334.8	10.8	0.181	0.126	0.035	0.161	81	+0.020
21....	328.8	9.7	0.162	0.119	0.031	0.150	81	+0.012
28....	333.4	9.8	0.163	0.110	0.028	0.138	83	+0.025
Dec. 5....	327.3	8.4	0.138	0.114	0.025	0.139	82	−0.001
12....	323.8	9.3	0.154	0.123	0.027	0.150	82	+0.004
19....	321.6	9.3	0.153	0.105	0.028	0.133	82	+0.020
26....	308.6	7.9	0.131	0.100	0.023	0.123	82	+0.008
1910.								
Jan. 2....	307.5	8.3	0.138	0.115	0.022	0.137	84	+0.001

These and other analytical data have been introduced in this paper in part reproduced in graphic form. In all of the charts the abscissa units represent days, and the ordinate units food (broken line) or body-weight (solid line). The food-intake curve is plotted from the total amount eaten per week. The average daily nitrogen balance is indicated as above (+) or below (—) the heavy line (o).

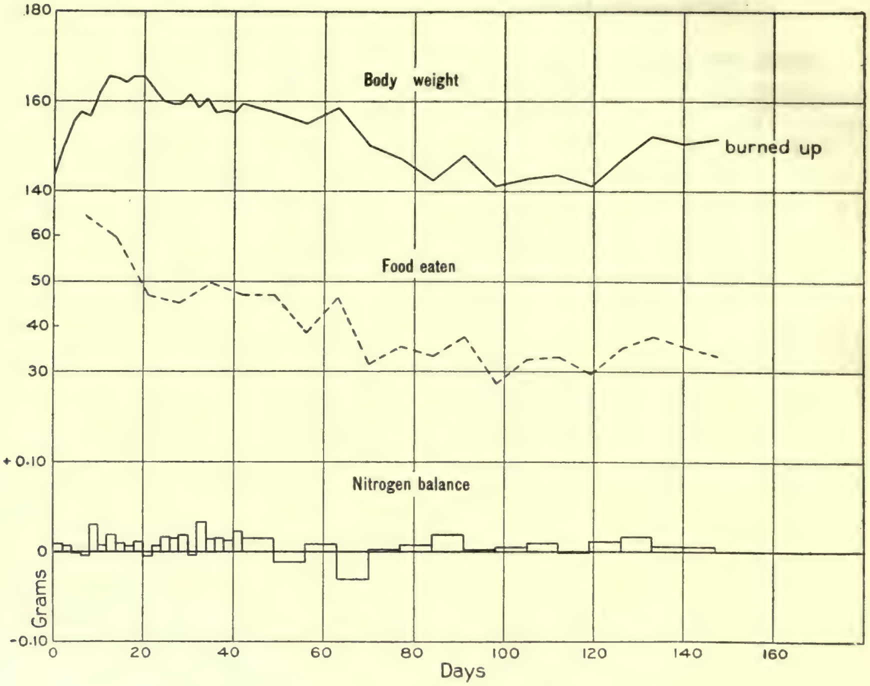

CHART II.—"Control" Rat XII fed on Dog Biscuit-Lard Diet for 147 days.

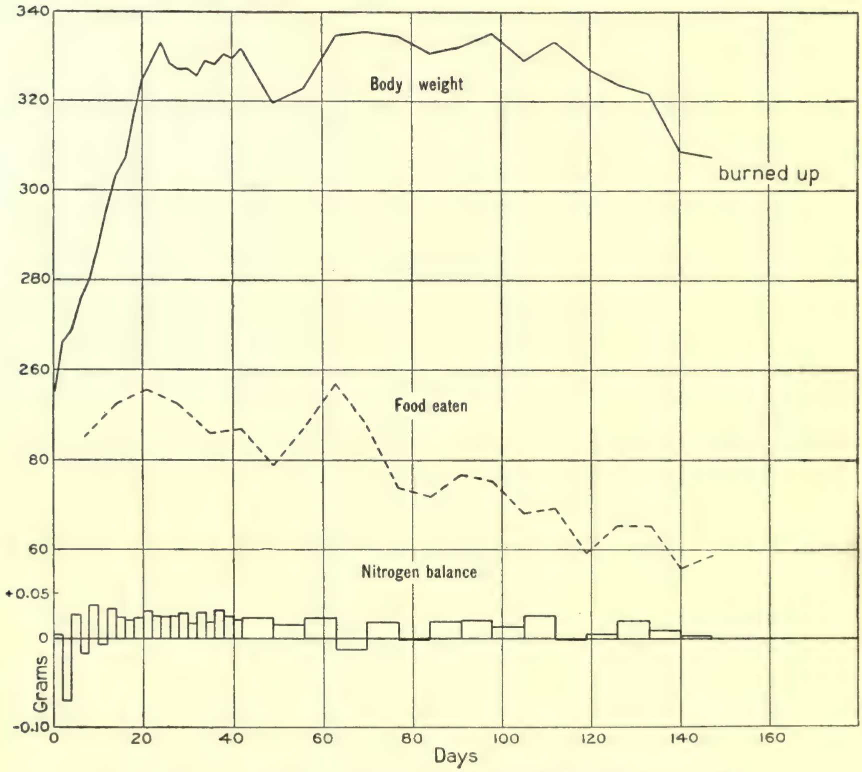

CHART III.—"Control" Rat XIII fed on Dog Biscuit-Lard Diet for 147 days.

DISCUSSION.

It will be noted that Rats XII and XIII, representing different ages, if we judge from the initial weights, were maintained in nutritive equilibrium without loss of body-weight during the period of 147 days—a not inconsiderable fraction of the life span of these animals. There is, however, a gradual decline in the amount of food which is eaten toward the end of the experiment, the quantity in the case of XII approaching limits which must have necessitated some demand upon the fat supply earlier accumulated. The utilization of the protein continued satisfactory, thus evincing unimpaired digestive powers It is far from likely that the ration used, with its large preponderance of energy in the form of fat, is an ideal one. The facts recorded, however, exclude the probability that monotony of diet is an insurmountable obstacle to nutritive success.*

As to the possibility of prolonged feeding on a uniform unchanged diet, two illustrations are appended of experiments on rats 14 and 18 fed with a mixture of ground hempseed, starch, lard, and salts. These rats were first fed with a mixture of dog biscuit and lard for several weeks and then on the hempseed mixture. The composition of the food given during the different periods is shown in table III.

TABLE III.—COMPOSITION OF THE FOOD IN PERCENTAGES.

	Dog-biscuit.	Lard.	Nitrogen.	Hemp-seed.	Starch.	Lard.	Sodium chloride.	Salt mixture I.†	Nitrogen.
Rat 14.									
Period 1....	58	42	1.6	....	....	....	....	....	....
Period 2....	70	30	1.9	....	....	....	....	....	....
Period 3....	....	....	....	46	42	10	2	....	2.27
Period 4....	....	....	....	46	42	10	....	2	2.40
Period 5....	....	....	....	50	38	10	....	2	2.32
Rat 18.									
Period 1....	58	42	1.6	....	....	....	....	....	....
Period 2....	70	30	1.9	....	....	....	....	....	....
Period 3....	....	....	....	46	42	10	2	....	2.27
Period 4....	....	....	....	46	42	10	....	2	2.39
Period 5....	....	....	....	50	38	10	....	2	2.45

The figures for nitrogen are averages of the different batches of food which were made up from time to time. The hempseed meal was freed from the greater part of the hulls by sifting, but the different lots contained different proportions which escaped separation; hence the actual nitrogen content of the individual batches of food varied somewhat. The figures given in the protocols and representing daily averages are based on the actual quantity of nitrogen fed, *not* on the averages given in table III.

*Among the many often unapparent difficulties which beset such experiments, the frequent occurrence of intestinal parasites and the susceptibility of the animals o digestive disturbances are to be noted.

†The salt mixture I, which contained organic and inorganic salts of the necessary bases and acids, is described on page 32.

Rat 14 lived 200 days without marked change in weight (losing less than 10 per cent); rat 18 (still on the same diet after 322 days at the time of writing) weighs very nearly as much as at the beginning of the hempseed feeding.

TABLE IV.—SUMMARY OF DATA ON RAT 14 FED ON HEMPSEED-STARCH-LARD DIET FOR 207 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
PERIOD I.								
Jan. 24....	284.0	.....	.....	.....	.....	.....	.....	.....
31....	274.2	8.5	0.134	0.123	0.029	0.152	78	−0.018
Feb. 7....	268.3	8.2	0.133	0.133	0.023	0.156	83	−0.023
PERIOD 2.								
Feb. 14....	262.5	8.7	0.168	0.137	0.032	0.169	81	−0.001
21....	255.4	7.4	0.143	0.147	0.027	0.174	81	−0.031
28....	257.0	9.3	0.178	0.145	0.032	0.177	82	+0.001
Mar. 7....	259.6	8.9	0.168	0.136	0.028	0.164	83	+0.004
PERIOD 3.								
Mar. 14....	208.0	.6	0.013	0.115	0.011	0.126	15	−0.113
21....	212.2	5.4	0.123	0.131	0.016	0.147	87	−0.024
PERIOD 4.								
Mar. 28....	234.2	9.7	0.231	0.144	0.044	0.188	81	+0.043
Apr. 4....	252.0	11.2	0.270	0.157	0.056	0.213	79	+0.057
18....	237.7	8.1	0.193	0.162	0.037	0.199	81	−0.006
May 2....	244.5	8.3	0.199	0.161	0.039	0.200	80	−0.001
16....	260.3	9.4	0.288	0.168	0.056	0.224	75	+0.004
30....	250.2	7.3	0.176	0.143	0.027	0.170	85	+0.006
June 6....	254.0	9.9	0.234	0.158	0.046	0.204	80	+0.030
20....	242.0	8.3	0.194	0.150	0.044	0.194	77	0.000
PERIOD 5.								
June 27....	251.8	8.5	0.205	0.142	0.039	0.181	81	+0.024
July 4....	248.0	8.1	0.200	0.155	0.026	0.181	87	+0.019
11....	247.4	8.4	0.208	0.156	0.043	0.199	79	+0.009
18....	251.5	8.7	0.210	0.127	0.045	0.172	79	+0.038
25....	253.0	8.9	0.213	0.148	0.040	0.188	81	+0.025
Aug. 1....	261.8	10.5	0.252	0.141	0.069	0.210	73	+0.042
8....	264.8	9.2	0.232	0.103	0.018	0.121	92	+0.111
15....	262.2	12.2	0.249	0.134	0.064	0.198	74	+0.051
22....	266.6	12.5	0.253	0.108	0.076	0.184	70	+0.069
29....	252.2	10.9	0.221	0.105	0.069	0.174	69	+0.047
Sept. 5....	249.5	11.8	0.262	0.141	0.077	0.218	71	+0.044
12....	241.5	9.5	0.219	0.174	0.044	0.218	80	+0.001
19....	245.0	13.5	0.314	0.215	0.101	0.316	68	−0.002
26....	237.8	10.1	0.235	0.201	0.051	0.252	78	−0.017
30.... Dead.	180.2							

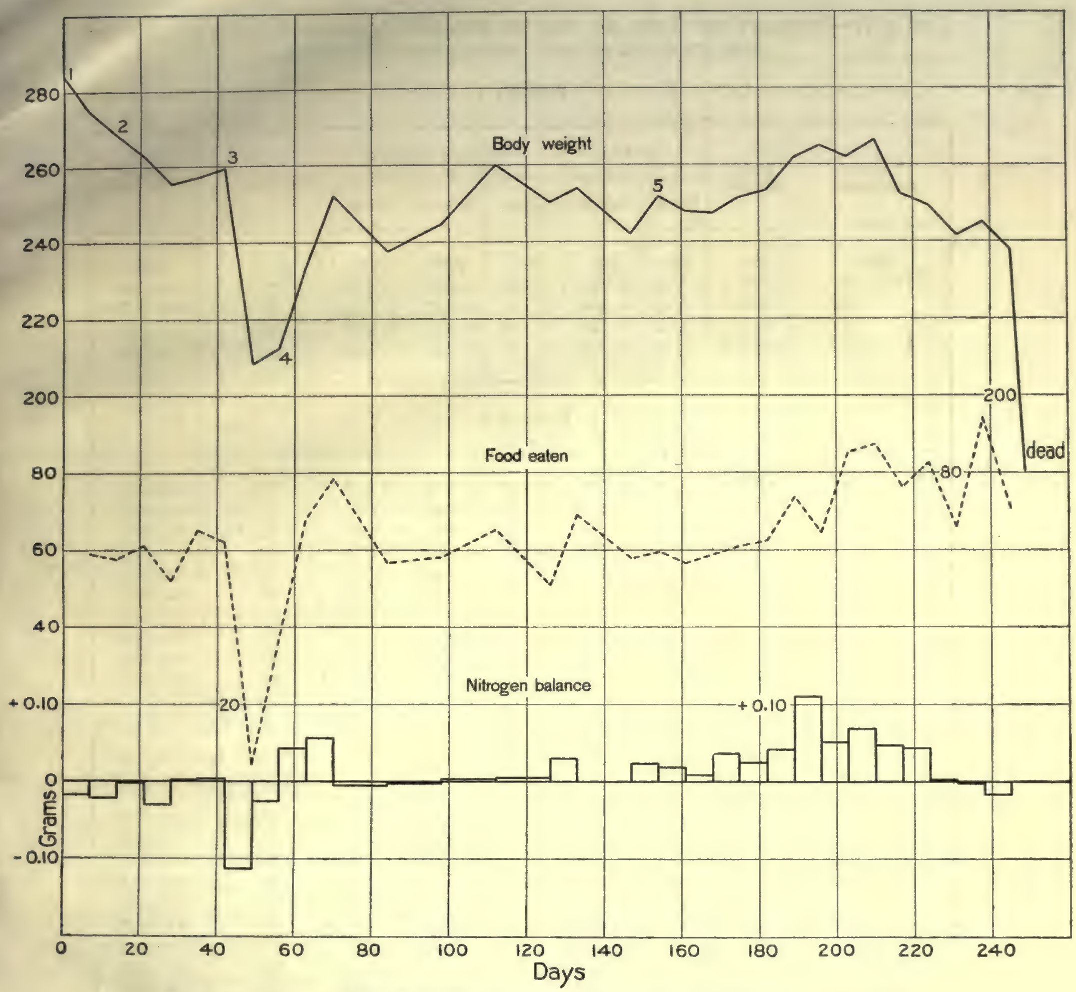

CHART IV.—Rat 14 fed on Hempseed-Lard Diet for 207 days. Numbers on body-weight line indicate time at which each period began.

TABLE V.—SUMMARY OF DATA ON RAT 18 FED ON HEMPSEED-STARCH-LARD DIET FOR 322 DAYS.—DAILY AVERAGES.

PERIOD 1.

Date of experiment.	Body-weight.	Intake. Food.	Intake. Nitrogen.	Nitrogen output. Urine.	Nitrogen output. Fæces.	Nitrogen output. Total.	N-utilization.	N-balance.
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Jan. 24....	268.3	......	....	....	....	......	....	....
31....	248.7	6.2	0.099	0.121	0.024	0.145	76	−0.046
Feb. 7....	233.5	4.9	0.078	0.095	0.015	0.110	81	−0.032
PERIOD 2.								
Feb. 14....	237.0	8.5	0.163	0.142	0.027	0.169	83	−0.006
21....	248.0	9.2	0.177	0.145	0.027	0.172	85	+0.005
28....	251.8	8.9	0.171	0.111	0.035	0.146	80	+0.025

TABLE V.—SUMMARY OF DATA ON RAT 18 FED ON HEMPSEED-STARCH-LARD DIET FOR 322 DAYS.—DAILY AVERAGES—CONTINUED.

PERIOD 3.

Date of experiment.	Body-weight.	Intakc.		Notiogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Mar. 7....	236.0	3.2	0.072	0.099	0.018	0.117	75	−0.045
14....	200.0	3.5	0.080	0.127	0.027	0.154	66	−0.074
21....	194.7	6.2	0.140	0.104	0.034	0.138	76	+0.002
28....	182.0	3.5	0.079	0.117	0.042	0.159	47	−0.080
PERIOD 4.								
Apr. 4....	168.2	4.2	0.098	0.131	0.018	0.149	82	−0.051
18....	159.0	5.4	0.129	0.135	0.028	0.163	78	−0.034
May 2....	175.5	6.7	0.161	0.114	0.026	0.140	84	+0.021
16....	216.0	11.8	0.287	0.118	0.060	0.178	79	+0.109
30....	230.0	7.7	0.187	0.107	0.032	0.139	83	+0.048
June 6....	229.0	8.5	0.205	0.102	0.041	0.143	80	+0.062
20....	242.0	8.2	0.195	0.109	0.046	0.155	76	+0.040
27....	235.0	5.8	0.135	0.104	0.027	0.131	80	+0.004
July 4....	235.5	8.0	0.188	0.127	0.036	0.163	81	+0.025
11....	243.8	9.3	0.230	0.138	0.041	0.179	82	+0.051
PERIOD 5.								
July 18....	243.1	7.2	0.177	0.119	0.039	0.158	78	+0.019
25....	242.5	7.6	0.183	0.139	0.035	0.174	81	+0.009
Aug. 1....	240.0	8.4	0.201	0.142	0.043	0.185	79	+0.016
8....	255.0	9.3	0.223	0.144	0.044	0.188	80	+0.035
15....	261.7	12.7	0.264	0.138	0.086	0.224	67	+0.040
22....	257.3	11.8	0.240	0.131	0.072	0.203	70	+0.037
29....	261.2	13.9	0.288	0.117	0.096	0.213	67	+0.075
Sept. 5....	256.0	10.5	0.240	0.137	0.086	0.223	64	+0.017
12....	239.0	8.8	0.203	0.189	0.044	0.233	78	−0.030
19....	234.0	11.8	0.275	0.193	0.085	0.278	69	−0.003
26....	215.8	7.3	0.171	0.191	0.037	0.228	78	−0.057
Oct. 3....	220.5	11.0	0.257	0.185	0.045	0.230	82	+0.027
10....	231.5	11.5	0.301	0.216	0.041	0.257	86	+0.044
17....	228.7	10.5	0.275	0.200	0.068	0.268	75	+0.007
24....	225.0	8.7	0.227	0.187	0.060	0.247	74	−0.020
31....	230.8	9.6	0.255	0.171	0.055	0.226	78	+0.029
Nov. 7....	230.0	10.2	0.281	0.185	0.076	0.261	73	+0.020
14....	227.0	7.6	0.209	0.173	0.048	0.221	77	−0.012
21....	218.7	8.3	0.229	0.196	0.042	0.238	82	−0.009
28....	222.5	8.5	0.233	0.164	0.037	0.201	84	+0.032
Dec. 5....	233.2	10.1	0.276	0.196	0.048	0.244	83	+0.032
12....	220.5	9.2	0.253	0.204	0.058	0.262	77	−0.009
19....	233.3	10.6	0.292	0.196	0.058	0.254	80	+0.038
26....	....	9.0	0.246	0.191	0.055	0.246	78	0.000
1911.								
Jan. 2....	239.0	9.7	0.253	0.174	0.058	0.232	77	+0.021
9....	232.7	6.6	0.164	0.143	0.024	0.167	85	−0.003
16....	239.8	7.7	0.191	0.148	0.024	0.172	87	+0.019

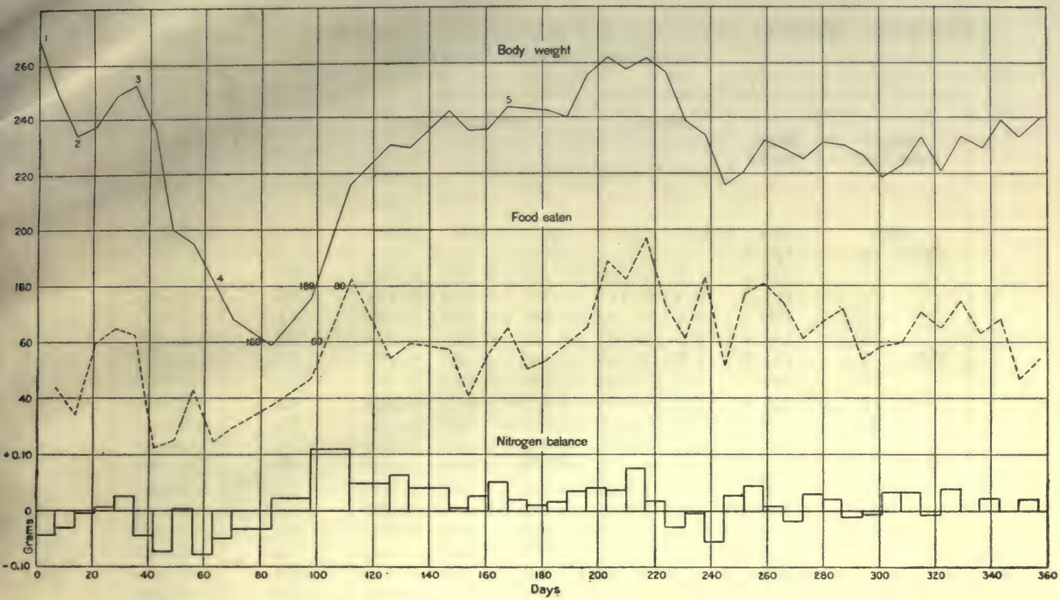

CHART V.—Rat 18 fed on Hempseed-Lard Diet for 322 days. Numbers on body-weight line indicate time at which each period began.

Other trials with the same diets frequently led to a decline in weight and a loss of body protein. In nearly all of these cases the insufficient food-intake was adequate to explain the incipient symptoms of inanition. Our numerous attempts to vary the flavor of the food and thus increase its palatability have been without striking success. Under exactly similar conditions of diet and environment different rats may continue to exhibit markedly unlike appetite for the same food. It seems best in the present stage of our knowledge to exclude from the diet experiments all animals which exhibit what seems like a temperamental anorexia. Protocols from some of these experiments are recorded here for comparison.

Rats XXVIII and XXIX were fed throughout the entire period on the dog biscuit-lard mixture with the results shown in tables VI and VII.

TABLE VI.—SUMMARY OF DATA ON RAT XXVIII FED ON DOG BISCUIT-LARD DIET FOR 105 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Sept. 19....	176.0	.....	.....	.....	.....	.....	.....	.....
21....	170.4	7.8	0.126	0.122	0.043	0.165	66	−0.039
23....	171.8	7.1	0.114	0.107	0.036	0.143	68	−0.029
25....	170.5	7.5	0.125	0.096	0.032	0.128	74	−0.003
30....	155.1	5.6	0.093	0.106	0.020	0.126	78	−0.033
Oct. 3....	152.8	5.8	0.096	0.096	0.026	0.122	73	−0.026
10....	152.5	5.9	0.095	0.082	0.019	0.101	80	−0.006
17....	147.5	5.1	0.083	0.075	0.018	0.093	78	−0.010
24....	148.5	6.9	0.114	0.080	0.022	0.102	81	+0.012
31....	141.3	4.8	0.080	0.067	0.012	0.079	85	+0.001
Nov. 7....	145.0	5.7	0.095	0.066	0.012	0.078	87	+0.017
14....	143.8	6.1	0.106	0.072	0.018	0.090	83	+0.016
21....	143.4	5.4	0.099	0.070	0.016	0.086	84	+0.013
28....	135.5	5.0	0.091	0.083	0.016	0.099	82	−0.008
Dec. 5....	134.5	5.3	0.097	0.080	0.017	0.097	82	0.000
12....	131.5	5.8	0.107	0.090	0.021	0.111	80	−0.004
19....	126.6	5.2	0.096	0.078	0.019	0.097	80	−0.001
26....	119.4	4.8	0.080	0.075	0.016	0.091	80	−0.011
1910.								
Jan. 2....	116.9	5.6	0.093	0.087	0.023	0.110	75	−0017

TABLE VII.—SUMMARY OF DATA ON RAT XXIX FED ON DOG BISCUIT-LARD DIET FOR 103 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Sept. 19....	175.0	.....	.....	.....	.....	.....	.....	.....
21....	170.2	8.6	0.138	0.138	0.038	0.176	72	−0.038
23....	174.2	8.8	0.141	0.127	0.035	0.162	75	−0.021
25....	....	8.3	0.137	0.103	0.032	0.135	77	+0.002
30....	163.3	6.6	0.107	0.107	0.025	0.132	77	−0.025
Oct. 3....	164.4	8.2	0.136	0.106	0.028	0.134	79	+0.002
10....	162.4	7.2	0.119	0.094	0.028	0.122	76	−0.003
17....	156.4	6.4	0.105	0.083	0.026	0.109	75	−0.004
24....	155.0	7.7	0.128	0.079	0.026	0.105	80	+0.023
31....	151.5	6.6	0.110	0.075	0.021	0.096	81	+0.014
Nov. 7....	150.6	6.9	0.116	0.079	0.025	0.104	78	+0.012
14....	151.0	6.2	0.111	0.075	0.018	0.093	84	+0.018
21....	153.4	6.9	0.125	0.077	0.023	0.100	82	+0.025
28....	150.3	7.8	0.142	0.076	0.023	0.099	84	+0.043
Dec. 5....	146.8	6.4	0.117	0.082	0.027	0.109	77	+0.008
12....	142.2	6.0	0.110	0.086	0.025	0.111	77	−0.011
19....	124.7	3.2	0.058	0.069	0.014	0.083	76	−0.025
26....	111.9	3.7	0.062	0.078	0.015	0.093	76	−0.031
31....	95.5	3.5	0.057	0.092	0.026	0.118	54	−0.061

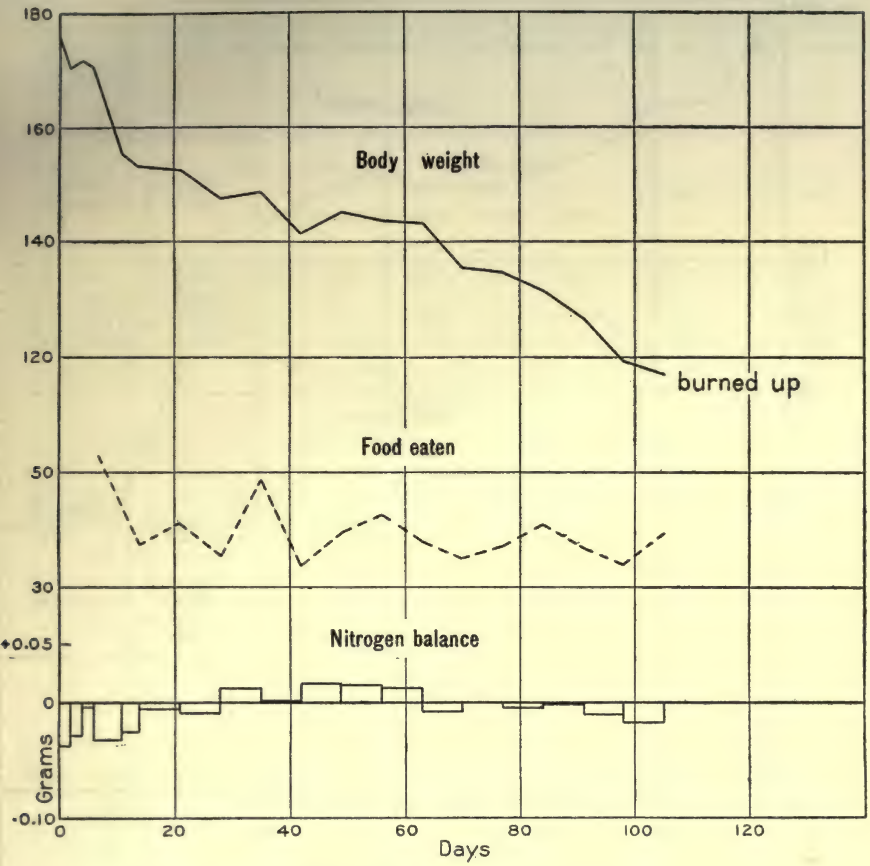

CHART VI.—Rat XXVIII fed on Dog Biscuit-Lard Diet for 105 days.

TABLE VIII.—COMPOSITION OF THE FOOD IN PERCENTAGES.

	Dog biscuit.	Lard.	Nitrogen.	Trumilk.*	Starch (arrowroot).	Lard.	Salt mixture I.	Nitrogen.
RAT 10.								
Period 1.....	58.0	42.0	1.58	......	......	......	......	......
Period 2.....	70.0	30.0	1.93	......	......	......	......	......
Period 3.....	......	......	......	54.0*†	16.0	30.0	......	2.51
Period 4.....	......	......	......	52.0*†	18.0	30.0	......	2.87
Period 5.....	......	......	......	42.0*†	24.0	34.0	......	2.31
RAT 11.								
Period 1.....	58.0	42.0	1.58	......	......	......	......	......
Period 2.....	70.0	30.0	1.93	......	......	......	......	......
Period 3.....	......	......	......	60.0	16.7	23.3	......	2.53
RAT 12.								
Period 1.....	58.0	42.0	1.58	......	......	......	......	......
Period 2.....	70.0	30.0	1.93	......	......	......	......	......
Period 3.....	......	......	......	60.0	16.7	23.3	......	2.53
Period 4.....	......	......	......	60.0	15.7	23.3	1.0	2.47

*"Trumilk" is a commercial milk powder.
†This was extracted in the laboratory once with 95 per cent alcohol, once with absolute alcohol, and four times with ether.

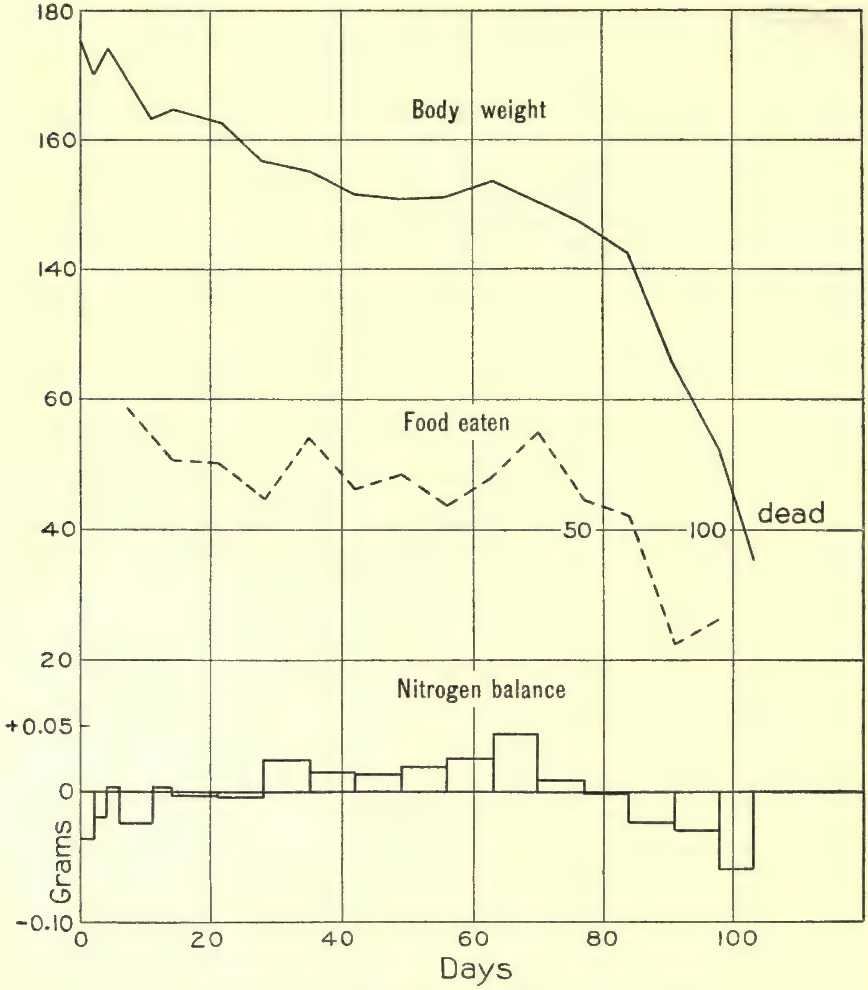

CHART VII.—Rat XXIX fed on Dog Biscuit-Lard Diet for 103 days.

Rats 10, 11, and 12 were first fed on the dog biscuit-lard mixture and later on one containing desiccated milk. The composition of their food is shown in table VIII.

TABLE IX.—SUMMARY OF DATA ON RAT 10 FED ON DOG BISCUIT-LARD DIET FOR 98 DAYS AND THEN ON MILK POWDER-STARCH-LARD DIET FOR 84 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
				PERIOD 1.				
1910.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Jan. 24....	237.6	......	......	......	......	......	....	....
31....	216.3	4.8	0.075	0.102	0.015	0.117	80	−0.042
Feb. 7....	210.0	5.7	0.090	0.107	0.014	0.121	84	−0.031
				PERIOD 2.				
Feb. 14....	208.0	7.9	0.151	0.127	0.029	0.156	81	−0.005
21....	211.3	8.1	0.155	0.129	0.027	0.156	83	−0.001
28....	212.7	6.9	0.132	0.105	0.022	0.127	83	+0.005
Mar. 7....	208.7	6.0	0.115	0.107	0.018	0.125	84	−0.010
14....	200.0	5.1	0.096	0.104	0.016	0.120	83	−0.024
21....	193.7	5.0	0.096	0.104	0.016	0.120	83	−0.024
Apr. 4....	186.5	5.7	0.112	0.098	0.018	0.116	84	−0.004
18....	177.5	5.6	0.106	0.103	0.014	0.117	87	−0.011
May 2....	182.8	6.4	0.120	0.101	0.018	0.119	85	+0.001
				PERIOD 3.				
May 9....	189.7	5.8	0.145	0.116	0.013	0.129	91	+0.016
16....	196.5	6.3	0.159	0.084	0.023	0.107	86	+0.052
23....	191.5	5.5	0.138	0.118	0.015	0.133	89	+0.005
				PERIOD 4.				
May 30....	185.0	5.1	0.134	0.134	0.014	0.148	90	−0.014
June 6....	175.5	5.9	0.169	0.169	0.019	0.188	89	−0.019
13....	172.1	6.4	0.183	0.167	0.013	0.180	93	+0.003
20....	176.2	4.6	0.132	0.146	0.011	0.157	92	−0.025
				PERIOD 5.				
June 27....	165.5	4.5	0.104	0.113	0.018	0.131	83	−0.027
July 4....	163.7	4.9	0.114	0.119	0.014	0.133	88	−0.019
11....	149.6	4.5	0.103	0.117	0.014	0.131	86	−0.028
18....	142.5	4.1	0.095	0.108	0.017	0.125	82	−0.030
25.... Chloroformed	134.3	4.4	0.104	0.131	0.022	0.153	79	−0.049

TABLE X.—SUMMARY OF DATA ON RAT 11 FED ON DOG BISCUIT-LARD DIET FOR 84 DAYS AND THEN ON MILK POWDER-STARCH-LARD DIET FOR 94 DAYS.—DAILY AVERAGES.

PERIOD 1.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Jan. 24....	224.3	......	......	......	......	......	......	......
31....	213.7	6.4	0.101	0.107	0.018	0.125	82	−0.024
Feb. 7....	208.8	7.3	0.116	0.116	0.020	0.136	83	−0.020

PERIOD 2.

Date of experiment.	Body-weight.	Food.	Nitrogen.	Urine.	Fæces.	Total.	N-utilization.	N-balance.
1910.								
Feb. 14....	196.7	6.3	0.120	0.115	0.020	0.135	83	−0.015
21....	202.5	7.4	0.142	0.126	0.018	0.144	87	−0.002
28....	195.0	6.4	0.123	0.120	0.023	0.143	81	−0.020
Mar. 7....	198.0	6.9	0.131	0.110	0.021	0.131	84	0.000
14....	187.3	5.3	0.100	0.102	0.015	0.117	85	−0.017
21....	190.1	5.1	0.100	0.102	0.018	0.120	82	−0.020
Apr. 4....	165.0	6.3	0.123	0.102	0.019	0.121	85	+0.002
18....	170.2	6.6	0.129	0.111	0.020	0.131	84	−0.002

PERIOD 3.

Date of experiment.	Body-weight.	Food.	Nitrogen.	Urine.	Fæces.	Total.	N-utilization.	N-balance.
1910.								
Apr. 25....	188.0	7.1	0.179	0.097	0.010	0.107	94	+0.072
May 2....	194.6	7.0	0.176	0.120	0.023	0.143	87	+0.033
9....	193.5	7.3	0.182	0.132	0.030	0.162	84	+0.020
16....	194.3	6.8	0.170	0.137	0.021	0.158	88	+0.012
23....	200.0	6.9	0.174	0.130	0.021	0.151	88	+0.023
30....	192.2	5.1	0.129	0.118	0.013	0.131	90	−0.002
June 6....	183.5	5.9	0.151	0.147	0.021	0.168	86	−0.017
13....	172.1	4.1	0.105	0.159	0.019	0.178	82	−0.073
20....	191.5	7.3	0.183	0.148	0.020	0.168	89	+0.015
27....	190.2	5.8	0.146	0.102	0.022	0.124	85	+0.022
July 4....	188.5	6.1	0.153	0.127	0.024	0.151	84	+0.002
11....	187.0	5.8	0.145	0.141	0.029	0.170	80	−0.025
18....	183.7	6.2	0.155	0.123	0.028	0.151	82	+0.004
21.... Dead	154.5							

TABLE XI.—SUMMARY OF DATA ON RAT 12 FED ON DOG BISCUIT-LARD DIET FOR 84 DAYS AND THEN ON MILK POWDER-STARCH-LARD DIET FOR 113 DAYS.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
PERIOD 1.								
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Jan. 24....	231.7	......	......	......	......	......	....	....
31....	212.0	5.7	0.091	0.096	0.021	0.117	77	−0.026
Feb. 7....	196.3	5.3	0.085	0.104	0.016	0.120	81	−0.035
PERIOD 2.								
1910.								
Feb. 14....	192.2	6.1	0.118	0.116	0.019	0.135	84	−0.017
21....	187.6	6.6	0.127	0.134	0.025	0.159	80	−0.032
28....	182.5	6.1	0.118	0.124	0.019	0.143	84	−0.025
Mar. 7....	181.3	5.5	0.104	0.114	0.012	0.126	88	−0.022
14....	182.2	7.1	0.134	0.117	0.020	0.137	85	−0.003
21....	184.6	7.3	0.141	0.108	0.015	0.123	89	+0.018
Apr. 4....	176.2	5.8	0.113	0.109	0.021	0.130	81	−0.017
18....	171.2	6.0	0.118	0.109	0.018	0.127	85	−0.009
PERIOD 3.								
1910.								
Apr. 25....	188.7	6.3	0.159	0.091	0.014	0.105	91	+0.054
May 2....	191.7	6.3	0.158	0.107	0.018	0.125	89	+0.033
9....	198.5	7.2	0.180	0.126	0.021	0.147	88	+0.033
16....	196.7	7.1	0.178	0.126	0.028	0.154	84	+0.024
23....	196.4	6.0	0.151	0.117	0.021	0.138	86	+0.013
30....	196.6	5.6	0.142	0.109	0.025	0.134	82	+0.008
June 6....	181.4	5.7	0.146	0.141	0.027	0.168	82	−0.022
13....	176.3	5.3	0.135	0.132	0.014	0.146	90	−0.011
20....	175.0	4.7	0.118	0.131	0.013	0.144	89	−0.026
27....	173.3	4.5	0.112	0.099	0.010	0.109	91	+0.003
July 4....	165.6	4.8	0.122	0.125	0.018	0.143	85	−0.021
11....	162.5	5.0	0.126	0.138	0.012	0.150	90	−0.024
18....	155.0	4.6	0.115	0.119	0.014	0.133	88	−0.018
25....	143.5	4.1	0.103	0.163	0.009	0.172	91	−0.069
PERIOD 4.								
1910.								
Aug. 1....	137.0	4.6	0.114	0.104	0.017	0.121	85	−0.007
8....	115.5	3.7	0.091	0.129	0.015	0.144	84	−0.053
9.... Dead	110.5							

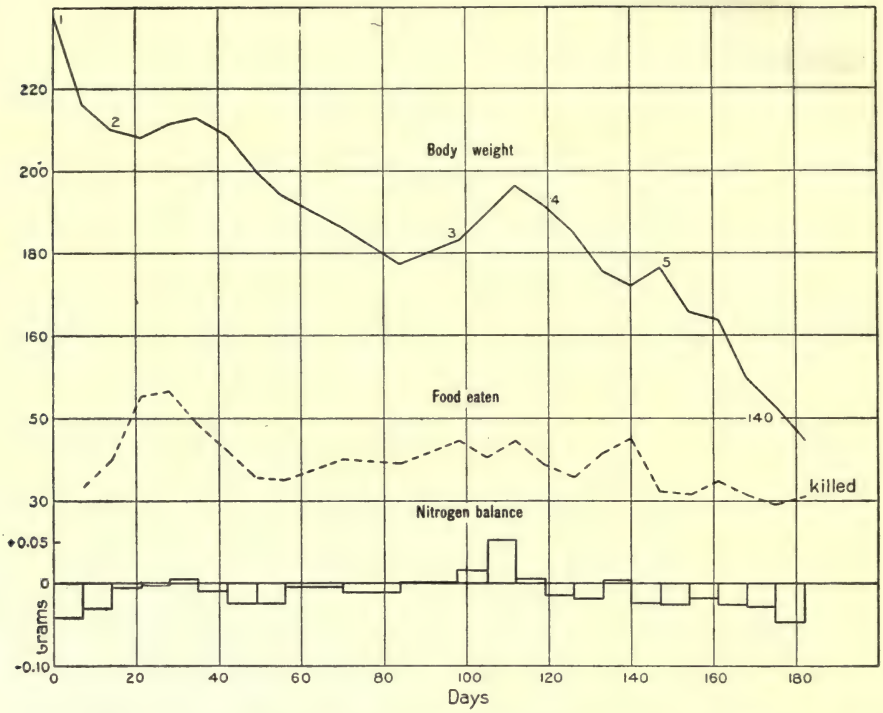

CHART VIII.—Rat 10 fed on Dog Biscuit-Lard Diet for 98 days and then on Milk powder-Starch-Lard Diet for 84 days. Numbers on Body-weight line indicate time at which each period began.

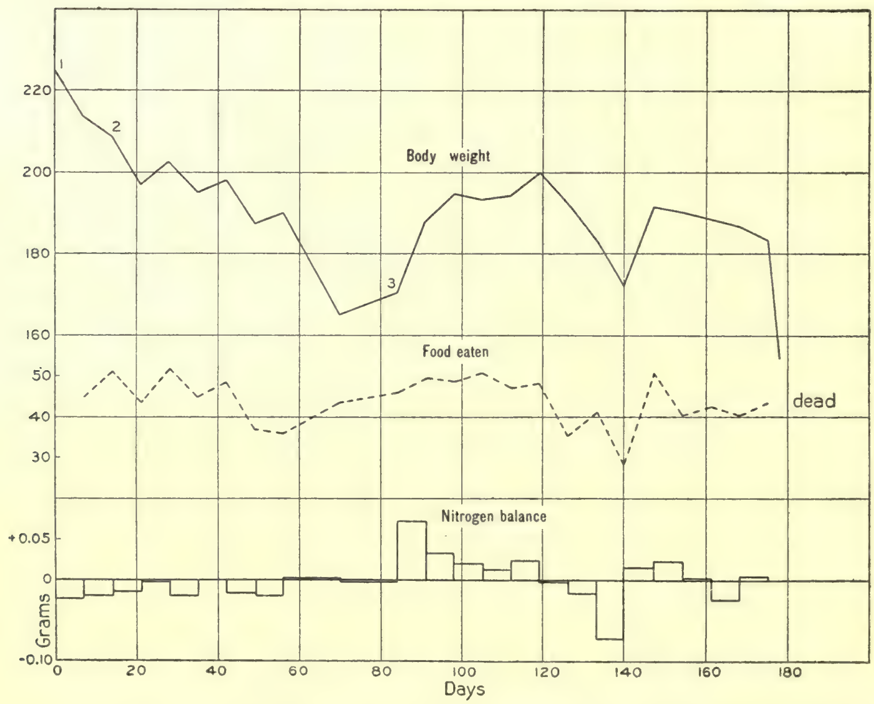

CHART IX.—Rat 11 fed on Dog Biscuit-Lard Diet for 84 days and then on Milk powder-Starch-Lard Diet for 94 days. Numbers on Body-weight line indicate time at which each period began.

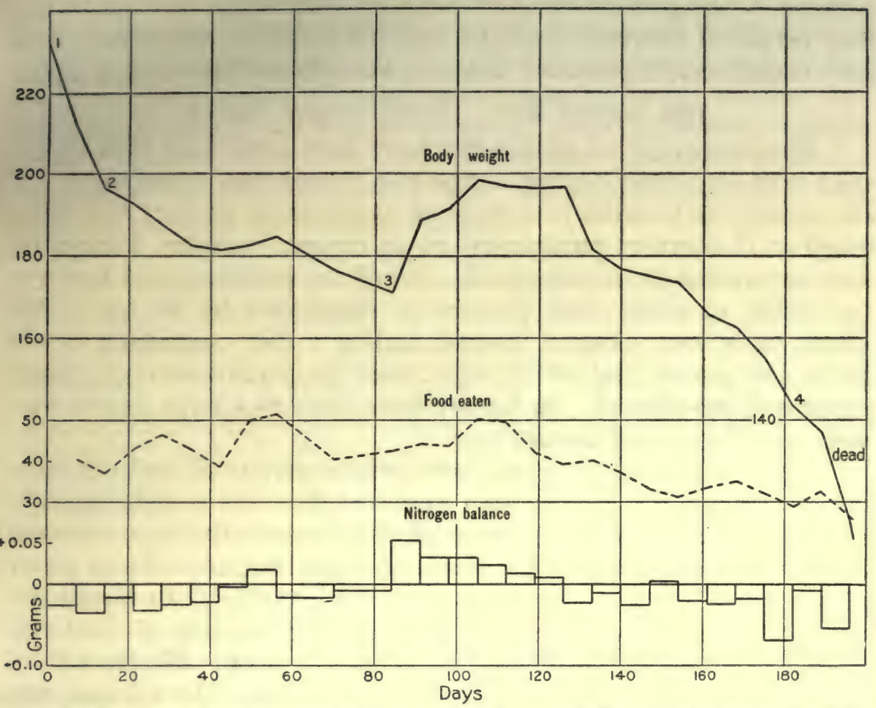

CHART X.—Rat 12, fed on Dog Biscuit-Lard Diet for 84 days and then on Milk powder-Starch-Lard Diet for 113 days. Numbers on Body-weight line indicate time at which each period began.

DISCUSSION.

The rats XXVIII and XXIX showed a steady decline on the dog-biscuit-fat mixture (p. 13). In considering the quantity of food eaten it must be borne in mind that the nitrogen content was rather low (N = 1.65 per cent).

Rats 10, 11, and 12 also showed, during the early period of the experiment, a steady decline on the same diet, although it contained more nitrogen. The temporary improvement shown after several weeks at the point marked 3 indicates the introduction of a change in diet. The improvement was, however, only temporary, as the charts indicate. The curves of body-weight in these animals recall those published by Falta and Noegerrath and correspond with the data of other investigators mentioned above. They indicate the type of experiment which is unsuccessful because of more or less obvious insufficiency in food-intake or stored supply. In the case of rats XXVIII and 10, for example, this is pronounced and a steady and continuous decline is noted. The decline of rat XXIX, at first gradual, became extremely marked with the striking decrease in the food-intake at the end of the experiment. The other illustrations (rats 11 and 12) show intermediate types. As a rule, older, full-grown animals exhibit slower decline than younger rats (of smaller weight)

because of the greater store of fat, etc. This helps to explain the long period of survival noted by various earlier investigators who have usually employed older (mature) animals for their feeding trials.

THE CASEIN DIET. PRELIMINARY TRIALS.

More experiments of this sort have been carried on with casein than with any other protein as the sole nitrogenous component. It can readily be isolated in a state of comparative purity; and inasmuch as it contains phosphorus in an organic complex, known by long experience to be assimilable, one of the problematical features pertaining to most other proteins is eliminated by its use. Our efforts have been directed toward finding a diet containing casein as the sole protein and which might meet the requirements of a long-continued experiment. In this we have been to a large degree successful in the case of mature rats.

Our earlier trials were conducted with a variety of food-mixtures containing casein. It was soon apparent that the protein requirement of the animals can be satisfied with comparative ease; accordingly the ration was prepared with a nitrogen concentration of about 2.5 per cent. The necessity for the use of much fat to insure the requisite paste consistency (and thus avoid scattering of the food) has put distinct limitations on the range of choice. We have tried without success to avoid the use of so much fat. To indicate the variety and proportion of inorganic elements which we have attempted to introduce, some of the mixtures are given in table XII.

TABLE XII.

Salt mixture I (Röhmann).	grams.	Salt mixture II (McCollum).	grams.	Salt mixture III.	grams.
$Ca_3(PO_4)_2$	10.0	Ash of milk	60.6	NaCl	33.4
K_2HPO_4	37.0	$Ca_3(PO_4)_2$	24.2	KCl	33.4
NaCl	20.0	NaCl	12.1	Bone ash	25.1
Na citrate	15.0	Fe citrate	3.1	Na_2CO_3	6.7
Mg citrate	8.0		100.0	Fe citrate	1.4
Ca lactate	8.0				100.0
Fe citrate	2.0				
	100.0				

It is still debatable whether any "roughage," such as cellulose, is absolutely necessary. McCollum* fed egg-yolk alone to white rats for 18 weeks without unfavorable results. Nevertheless we have introduced an indigestible residue as conforming more nearly to the usual alimentary experience of the animals; and agar-agar was selected because it is more easily manipulated than cellulose and because experience with other animals has shown us how efficient it is for this purpose.†

*McCollum: American Journal of Physiology, 1909, xxv, p. 127.

†Cf. Saiki: Journal of Biological Chemistry, 1906, II, p. 251. Swartz: Transactions of the Connecticut Academy of Arts and Sciences, 1911, xvi, p. 247. Mendel and Swartz: American Journal of Medical Sciences, March, 1910.

In order to compare the suitability of casein diets containing the salt mixtures recorded above, a series of trials was begun on rats which had previously been under observation in cages so that any eccentricity of eating or of metabolism might be noted. During this preliminary period the diet consisted of the dog biscuit mixture already referred to in the "control" series. During the casein periods the following diets were used, which are indicated in the tables by the corresponding numbers.

TABLE XIII.

	1	2	3	4	5	6
Pure casein	13.4	18.0	10.0	18.0	12.0	18.0
Cane sugar	20.6	15.0	4.0	15.0	15.0	15.0
Starch (arrow-root)	23.7	29.5	46.0	29.5	30.0	29.5
Lard	35.0	30.0	30.8	30.0	30.0	30.0
Agar	5.2	5.0	4.4	5.0	5.0	5.0
Salt mixture I	....	....	4.8	2.5	....	....
Salt mixture II	....	....	....	....	8.0	2.5
Salt mixture III	2.1	2.5	....	....	....	....
	100.0	100.0	100.0	100.0	100.0	100.0
Nitrogen	1.87	2.51	1.38	2.54	1.54	2.53

The records of the animals during the entire course of the experiment, until it was stopped by the loss of the laboratory by fire, are shown in Tables XIV–XXII and Charts XI–XIII.

TABLE XIV.—SUMMARY OF DATA ON RAT XXX, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE III FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 1	159.0	......	......	......	......	......	......	......
8	178.8	9.8	0.158	0.139	0.019	0.158	88	0.000
15	191.3	10.8	0.180	0.119	0.032	0.151	82	+0.029
22	185.6	9.3	0.154	0.114	0.028	0.142	82	+0.012
PERIOD 2.—CASEIN DIET 1.								
1909.								
Nov. 29	167.0	5.1	0.096	0.091	0.023	0.114	76	−0.018
Dec. 6	166.1	6.0	0.112	0.091	0.020	0.111	82	+0.001
13	162.7	5.4	0.101	0.091	0.015	0.106	85	−0.005
PERIOD 3.—CASEIN DIET 2.								
1909.								
Dec. 20	164.5	5.7	0.144	0.123	0.015	0.138	90	+0.006
27	170.1	6.5	0.164	0.139	0.014	0.153	91	+0.011
1910.								
Jan. 3	174.3	7.1	0.180	0.149	0.017	0.166	91	+0.014

TABLE XV.—SUMMARY OF DATA ON RAT XXXI, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE III FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 1....	181.7	......	......	......	......	......	......	......
8....	204.2	10.8	0.173	0.140	0.025	0.165	86	+0.008
15....	204.5	8.2	0.136	0.107	0.011	0.118	92	+0.018
22....	197.1	7.7	0.128	0.113	0.024	0.137	81	−0.009
				PERIOD 2.—CASEIN DIET 1.				
1909.								
Nov. 29....	184.1	4.7	0.089	0.089	0.018	0.107	80	−0.018
Dec. 6....	186.1	6.9	0.129	0.089	0.022	0.111	83	+0.018
13....	199.7	9.0	0.168	0.092	0.033	0.125	80	+0.043
				PERIOD 3.—CASEIN DIET 2.				
1909.								
Dec. 20....	208.5	7.9	0.199	0.111	0.023	0.134	82	+0.065
27....	202.7	9.4	0.237	0.160	0.026	0.186	89	+0.051
1910.								
Jan. 3....	198.5	6.5	0.164	0.146	0.030	0.176	82	−0.012

TABLE XVI.—SUMMARY OF DATA ON RAT XXXII, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE III FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 1....	180.5	......	......	......	......	......	......	......
8....	196.7	9.3	0.148	0.145	0.017	0.162	89	−0.014
15....	200 3	7.4	0.120	0.099	0.020	0.119	83	+0.001
22....	201.4	9.2	0.152	0.111	0.034	0.145	78	+0.007
				PERIOD 2.—CASEIN DIET 1.				
1909.								
Nov. 29....	193.7	6.1	0.113	0.095	0.018	0.113	84	0.000
Dec. 6....	189.6	5.8	0.109	0.089	0.020	0.109	82	0.000
13....	191.2	7.1	0.133	0.093	0.024	0.117	82	+0.016
				PERIOD 3.—CASEIN DIET 2.				
1909.								
Dec. 20....	193.8	6.1	0.153	0.113	0.015	0.128	90	+0.025
27....	199.3	7.6	0.192	0.154	0.018	0.172	91	+0.020
1910.								
Jan. 3....	204.7	7.8	0.198	0.160	0.020	0.180	90	+0.018

TABLE XVII.—SUMMARY OF DATA ON RAT XXXIII, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE I FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 1....	206.0	......	......	......	......	......	......	......
8....	220.2	8.4	0.135	0.138	0.023	0.161	83	−0.026
15....	226.3	8.6	0.137	0.116	0.021	0.137	85	0.000
22....	220.2	8.2	0.136	0.127	0.026	0.153	81	−0.017
PERIOD 2.—CASEIN DIET 3.								
1909.								
Nov. 29....	205.5	5.6	0.077	0.086	0.023	0.109	70	−0.032
Dec. 6....	199.2	6.9	0.095	0.071	0.042	0.113	56	−0.018
PERIOD 3.—CASEIN DIET 4.								
1909.								
Dec. 13....	206.3	7.5	0.156	0.092	0.034	0.126	78	+0.030
20....	219.5	8.8	0.223	0.102	0.033	0.135	85	+0.088
27....	226.8	9.5	0.240	0.165	0.040	0.205	83	+0.035
1910.								
Jan. 3....	233.0	9.4	0.235	0.168	0.015	0.183	94	+0.052

TABLE XVIII.—SUMMARY OF DATA ON RAT XXXIV, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE I FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 8....	157.0	......	......	......	......	......	......	......
15....	170.4	9.3	0.157	0.124	0.030	0.154	81	+0.003
22....	169.3	8.2	0.137	0.092	0.030	0.122	78	−0.015
PERIOD 2.—CASEIN DIET 3.								
1909.								
Nov. 29....	164.2	5.9	0.081	0.067	0.017	0.084	79	−0.003
Dec. 6....	157.5	5.9	0.081	0.064	0.024	0.088	70	−0.007
PERIOD 3.—CASEIN DIET 4.								
1909.								
Dec. 13....	172.5	8.0	0.172	0.080	0.036	0.116	79	+0.056
20....	181.8	7.8	0.199	0.108	0.039	0.147	80	+0.052
27....	198.5	9.4	0.236	0.127	0.039	0.166	83	+0.070
1910.								
Jan. 3....	206.2	9.0	0.226	0.125	0.047	0.172	79	+0.054

TABLE XIX.—SUMMARY OF DATA ON RAT XXXV, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE I FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 8....	116.5	......	......	......	......	......	......	......
15....	122.7	6.5	0.110	0.101	0.020	0.121	82	−0.011
22....	126.2	6.2	0.102	0.074	0.017	0.091	83	+0.011
PERIOD 2.—CASEIN DIET 3.								
1909.								
Nov. 29....	111.4	4.0	0.055	0.052	0.020	0.072	64	−0.017
Dec. 6....	118.3	4.5	0.062	0.050	0.014	0.064	77	−0.002
PERIOD 3.—CASEIN DIET 4.								
1909.								
Dec. 13....	127.1	6.1	0.126	0.060	0.028	0.088	78	+0.038
20....	142.9	8.8	0.225	0.105	0.025	0.130	89	+0.095
27....	147.3	7.2	0.181	0.116	0.029	0.145	84	+0.036
1910.								
Jan. 3....	152.5	6.7	0.167	0.108	0.026	0.134	84	+0.033

TABLE XX.—SUMMARY OF DATA ON RAT XXXVII, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE II FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Nov. 8....	142.7	......	......	......	......	......	......	......
15....	153.2	8.7	0.146	0.115	0.023	0.138	84	+0.008
22....	156.3	7.8	0.128	0.091	0.026	0.117	80	+0.011
PERIOD 2.—CASEIN DIET 5.								
1909.								
Nov. 29....	136.7	4.6	0.070	0.072	0.024	0.096	66	−0.026
Dec. 6....	136.0	5.7	0.088	0.071	0.034	0.105	61	−0.017
PERIOD 3.—CASEIN DIET 6.								
1909.								
Dec. 13....	135.5	5.6	0.116	0.085	0.025	0.110	78	+0.006
20....	137.5	7.3	0.184	0.109	0.021	0.130	89	+0.054
27....	140.8	6.2	0.159	0.122	0.024	0.146	85	+0.013
1910.								
Jan. 3....	143.3	5.9	0.151	0.121	0.021	0.142	86	+0.009

TABLE XXI.—SUMMARY OF DATA ON RAT XL, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE II FOR 42 DAYS.—DAILY AVERAGES.

PERIOD 1.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Nov. 10....	150.0	......	......	......	......	......	....	....
15....	135.4	2.7	0.045	0.106	0.007	0.113	84	−0.068
22....	147.3	7.7	0.130	0.096	0.021	0.117	84	+0.013
PERIOD 2.—CASEIN DIET 5.								
1909.								
Nov. 29....	137.8	4.6	0.071	0.059	0.020	0.079	82	−0.008
Dec. 6....	138.5	6.3	0.100	0.068	0.033	0.101	67	−0.001
PERIOD 3.—CASEIN DIET 6.								
1909.								
Dec. 13....	139.5	6.1	0.129	0.088	0.027	0.115	79	+0.014
20....	130.0	4.3	0.109	0.099	0.019	0.118	83	−0.009
27....	115.6	3.4	0.087	0.102	0.014	0.116	84	−0.029
1910.								
Jan. 3....	110.0	4.0	0.103	0.105	0.017	0.122	83	−0.019

TABLE XXII.—SUMMARY OF DATA ON RAT XLII, FED ON PURE CASEIN (AS THE ONLY PROTEIN) AND SALT MIXTURE II FOR 42 DAYS.—DAILY AVERAGES.

PERIOD I.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1909.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Nov. 10....	144.0	......	......	......	......	......	....	....
15....	137.8	4.9	0.082	0.090	0.013	0.103	84	−0.021
22....	141.4	6.3	0.106	0.069	0.020	0.089	81	+0.017
PERIOD 2.—CASEIN DIET 5.								
1909.								
Nov. 29....	130.2	3.8	0.059	0.062	0.019	0.081	68	−0.022
Dec. 6....	130.6	5.6	0.086	0.072	0.031	0.103	64	−0.017
PERIOD 2.—CASEIN DIET 6.								
1909.								
Dec. 13....	126.4	4.8	0.101	0.086	0.022	0.108	78	−0.007
20....	128.4	5.0	0.126	0.105	0.021	0.126	83	0.000
27....	122.4	4.7	0.120	0.108	0.023	0.131	81	−0.011
1910.								
Jan. 3....	116.2	4.4	0.113	0.109	0.025	0.134	78	−0.021

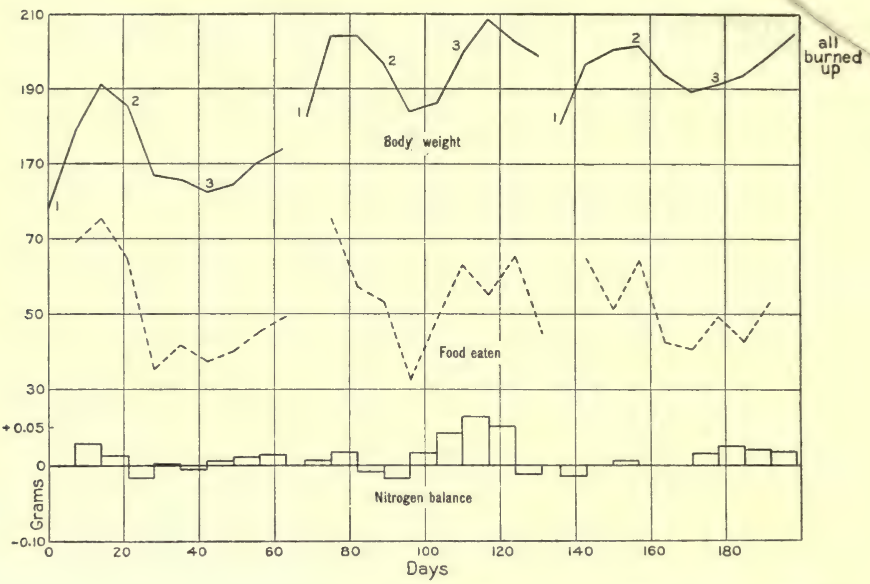

CHART XI.—Rats XXX, XXXI, and XXXII fed on pure Casein as the only protein and Salt mixture III for 42 days. Numbers on Body-weight line indicate the time at which each period began.

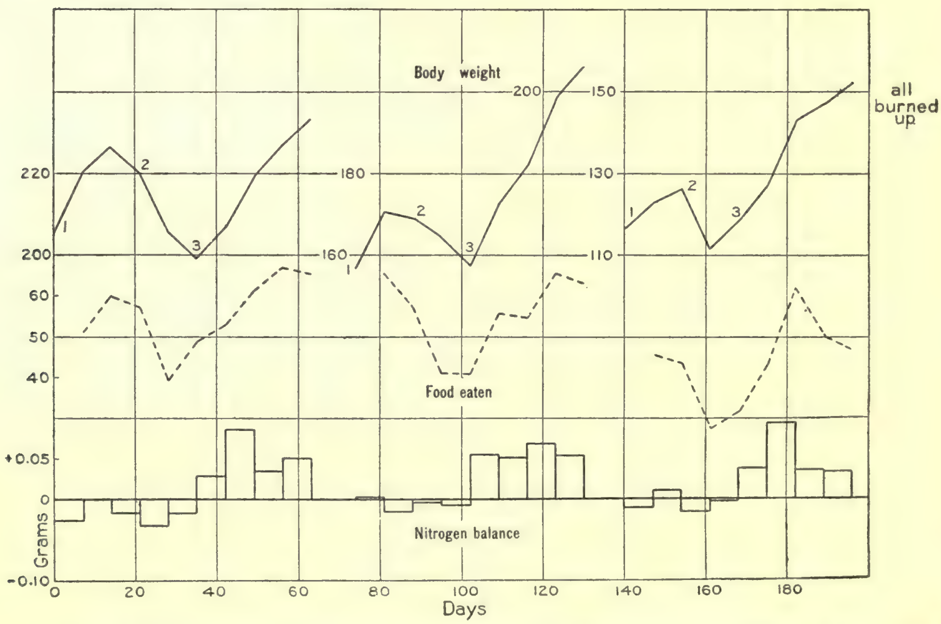

CHART XII.—Rats XXXIII, XXXIV, and XXXV fed on pure Casein as the only protein and Salt mixture I for 42 days. Numbers on Body-weight line indicate time at which each period began.

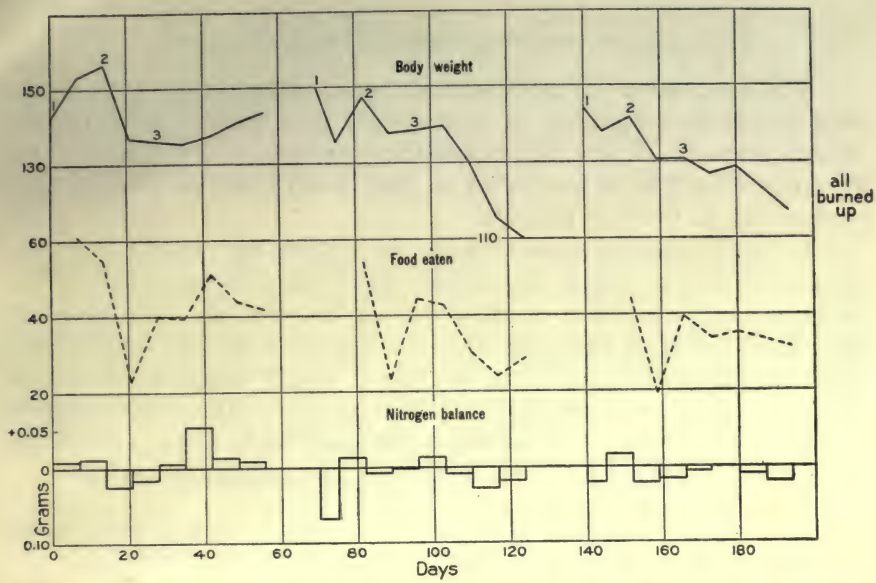

CHART XIII.—Rats XXXVII, XL, and XLII fed on pure Casein as the only protein and Salt Mixture II for 42 days. Numbers on Body-weight lines indicate time at which each period began.

DISCUSSION.

The essential differences in these series lasting 42 days involve the inorganic constituents of the diet. All of the animals selected, with two exceptions (XL and XLII)* gave a fairly satisfactory record during a three weeks preliminary trial, whereupon they were selected for the casein feeding. It will be seen that the most promising nutritive conditions were afforded by mixture I, fed to rats XXXIII, XXXIV, and XXXV, in which the inorganic constituents were closely copied after Röhmann's successful ash mixture, with the addition of a little iron. During the first two weeks of casein feeding these three rats lost weight and nitrogen. This was caused by diarrhœa due to too great a proportion of inorganic salts in the food mixture. When this was reduced from 4.8 to 2.5 per cent a very rapid gain in weight and nitrogen at once took place. The superiority of the food mixture given to these three rats in contrast with the other two mixtures given to the other rats is plainly evident from comparison of these data. These experiments lasted 42 days and showed distinct gains in the nitrogen balance and also in body-weight during the period mentioned.†

*These two animals were not pure white rats, but partly colored. They ate very poorly. We have gained the impression from observations on a large number of rats that these hybrid forms are not suited to our experimental needs and therefore have lately employed only the pure white races.

†It may be noted that the *apparently* poorer utilization of nitrogen during some of the periods when little food was eaten is in part attributable to the fact that output of nitrogenous alimentary secretions continues despite the smaller intake of nitrogen.

THE CASEIN DIET—PROLONGED FEEDING TRIALS.

The proportions of this food mixture (4, see page 33) thus tested were therefore employed in subsequent experiments as a typical "basal" ration. We record below the protocols of a few rats which for many weeks have been kept in good health on this dietary with pure casein as the sole protein.

By an ill-advised plan these casein-fed rats were transferred during one month to cages containing sand, and the estimation of the nitrogenous excreta was omitted during this period. It was subsequently discovered that the animals ate more or less sand during this time and we attribute the decline of weight and the subsequent death in some cases to a resulting damage of the alimentary organs, since sand was found in the fæces of some of the rats long afterwards and autopsy showed great congestion of the intestinal tract.

TABLE XXIII.—SUMMARY OF DATA ON RAT 23, FED FOR 135 DAYS ON PURE CASEIN AS THE ONLY PROTEIN.—DIET 4, PAGE 33.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Feb. 7....	195.7							
14....	209.3	8.0	0.208	0.111	0.026	0.137	87	+0.071
21....	222.0	9.4	0.247	0.118	0.041	0.159	83	+0.088
28....	220.5	8.1	0.215	0.108	0.039	0.147	82	+0.068
Mar. 7....	212.8	7.0	0.186	0.141	0.031	0.172	83	+0.014
14....	210.0	6.6	0.173	0.145	0.024	0.169	86	+0.004
28....	221.1	7.9	0.209	0.155	0.029	0.184	86	+0.025
Apr. 11....	232.0	7.9	0.208	0.147	0.030	0.177	86	+0.031
18....	234.4	9.1						
25....	217.6	5.9						
May 2....	201.8	4.7						
9....	211.3	5.4						
16....	219.0	7.2	0.185	0.112	0.027	0.139	85	+0.046
23....	225.0	7.7	0.197	0.137	0.028	0.165	86	+0.032
30....	220.8	6.6	0.168	0.131	0.026	0.157	85	+0.011
June 6....	225.6	7.9	0.201	0.140	0.029	0.169	86	+0.032
13....	212.3	6.2	0.158	0.155	0.025	0.180	84	−0.022
20....	197.0	4.9	0.126	0.146	0.017	0.163	87	−0.037
22....	182.7							
Dead								

TABLE XXIV.—SUMMARY OF DATA ON RAT 24, FED FOR 169 DAYS ON PURE CASEIN AS THE ONLY PROTEIN.—DIET 4, PAGE 33.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Feb. 7....	227.0	......	......	......	......	......	......	......
14....	239.0	8.6	0.226	0.145	0.023	0.168	90	+0.058
21....	244.0	10.1	0.266	0.180	0.039	0.219	85	+0.047
28....	239.0	9.4	0.248	0.192	0.037	0.229	85	+0.019
Mar. 7....	241.0	8.2	0.217	0.180	0.022	0.202	90	+0.015
14....	242.0	8.8	0.234	0.181	0.032	0.213	86	+0.021
28....	259.6	9.6	0.254	0.195	0.031	0.226	88	+0.028
Apr. 11....	252.7	9.0	0.235	0.176	0.032	0.208	86	+0.027
18....	235.8	6.5	......	......	......	......	......	......
25....	218.8	4.6	......	......	......	......	......	......
May 2....	205.2	4.5	......	......	......	......	......	......
9....	214.8	5.8	......	......	......	......	......	......
16....	216.1	7.0	0.178	0.123	0.029	0.152	84	+0.026
23....	240.5	9.5	0.242	0.150	0.030	0.180	88	+0.062
30....	252.4	9.1	0.233	0.163	0.023	0.186	90	+0.047
June 6....	257.1	8.8	0.226	0.161	0.024	0.185	89	+0.041
13....	248.7	7.3	0.188	0.163	0.021	0.184	89	+0.004
20....	243.6	6.7	0.172	0.155	0.018	0.173	90	−0.001
27....	246.6	6.4	0.164	0.142	0.014	0.156	91	+0.008
July 4....	243.5	7.3	0.184	0.152	0.018	0.170	90	+0.014
11....	244.1	8.2	0.209	0.166	0.031	0.197	85	+0.012
18....	230.0	8.6	0.220	0.133	0.049	0.182	78	+0.038
25....	169.1	1.6	0.042	0.103	0.037	0.140	12	−0.098
26....	157.3							
Chlorof'd.....								

TABLE XXV.—SUMMARY OF DATA ON RAT 25, FED FOR 176 DAYS ON PURE CASEIN AS THE ONLY PROTEIN.—DIET 4, PAGE 33.—DAILY AVERAGES.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Feb. 7....	177.0	......	......	......	......	......	......	......
14....	189.0	9.6	0.252	0.170	0.034	0.204	87	+0.048
21....	184.6	7.8	0.207	0.164	0.033	0.197	84	+0.010
28....	199.5	9.3	0.245	0.176	0.021	0.197	91	+0.048
Mar. 7....	198.3	9.0	0.238	0.175	0.033	0.208	86	+0.030
14....	199.2	7.9	0.210	0.181	0.029	0.210	86	0.000
28....	199.0	7.9	0.210	0.176	0.026	0.202	88	+0.008
Apr. 11....	198.2	7.5	0.196	0.159	0.031	0.190	84	+0.006
18....	200.6	9.1	......	......	......	......	......	......
25....	187.2	5.4	......	......	......	......	......	......
May 2....	167.3	3.9	......	......	......	......	......	......
9....	179.2	6.3	......	......	......	......	......	......
16....	181.0	6.3	0.161	0.125	0.020	0.145	88	+0.016
23....	186.0	7.1	0.180	0.126	0.029	0.155	84	+0.025
30....	190.5	7.2	0.185	0.138	0.024	0.162	87	+0.023
June 6....	196.1	8.5	0.219	0.149	0.022	0.171	90	+0.048
13....	191.0	7.1	0.181	0.154	0.024	0.178	87	+0.003
20....	185.0	6.3	0.160	0.146	0.023	0.169	86	−0.009
27....	185.0	6.2	0.157	0.124	0.023	0.147	85	+0.010
July 4....	177.6	4.9	0.125	0.106	0.025	0.131	80	−0.006
11....	165.8	3.7	0.095	0.098	0.015	0.113	84	−0.018
18....	153.5	3.1	0.078	0.076	0.014	0.090	82	−0.012
25....	140.5	3.3	0.084	0.090	0.017	0.107	80	−0.023
Aug. 1....	110.0	2.0	0.052	0.086	0.018	0.104	65	−0.052
Dead 2....	107.0							

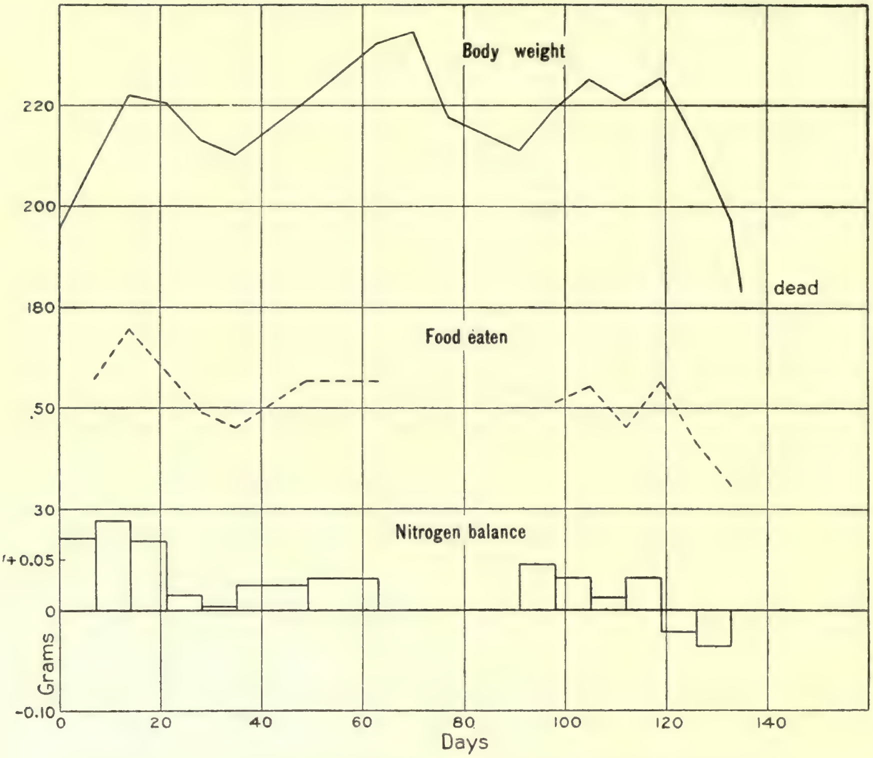

CHART XIV.—Rat 23 fed 135 days on pure Casein as the only protein, Diet 4, p. 33.

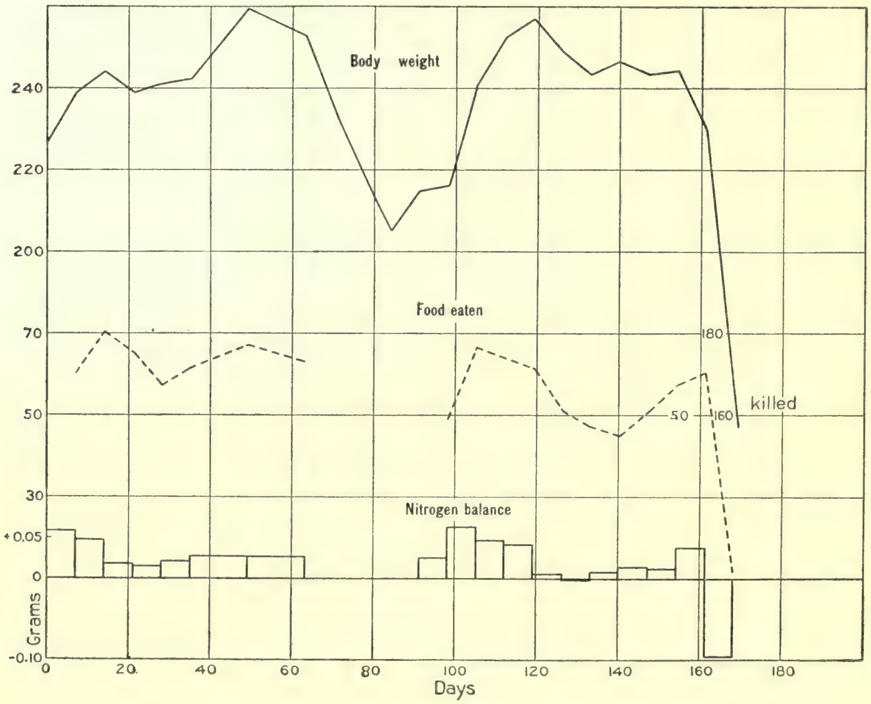

CHART XV.—Rat 24 fed 169 days on pure Casein as the only protein, Diet 4, p. 33.

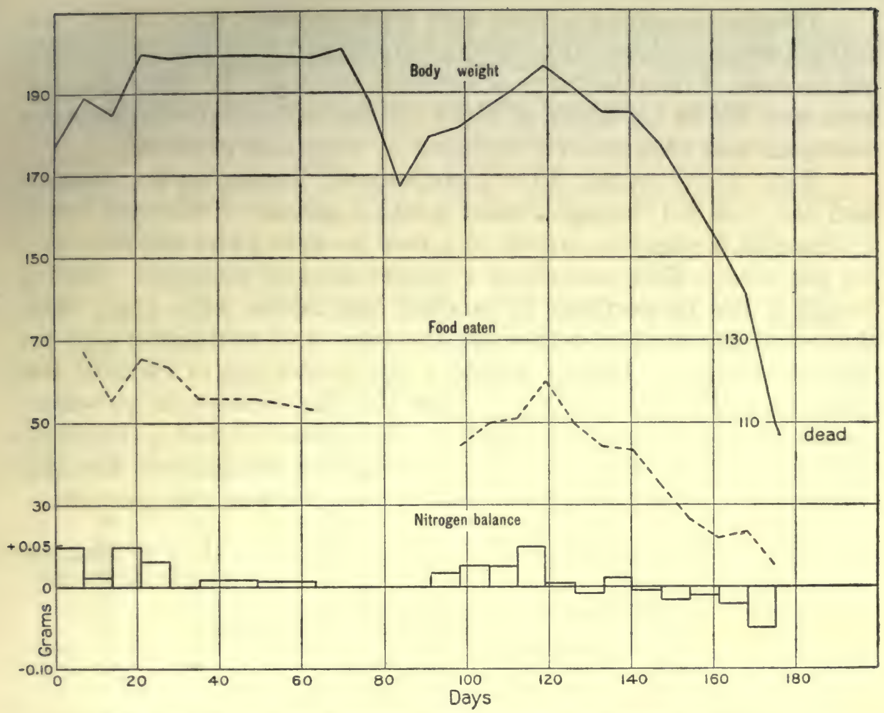

CHART XVI.—Rat 25 fed 176 days on pure Casein as the only protein, Diet 4, p. 33.

These experiments, with rats 23, 24 and 25, extending over 135–160 days without noteworthy alterations in the weight and with a very large gain of nitrogen, are, as far as we are aware, the most successful recorded attempts at artificial nutrition with a constant mixture of pure foodstuffs including a single protein.

CASEIN AND VEGETABLE PROTEIN DIET.

Without reporting here the numerous trials and failures to replace part or all of the casein by other proteins, a few data from our records may throw light upon the difficulties thus encountered. The proportion of the nutrients is the same in these experiments, one-third or more of the casein being replaced by the proteins indicated.

A casual inspection of the succeeding pages shows that the failure to eat is frequently sufficient to account for the failure to maintain body-weight and tissue. The animals lost weight to the extent of their fat content and then speedily succumbed with indications of inanition rather than any specific pathological metabolism. Those rats which ate less than 40 grams of the mixed food were unable to maintain their nutritive equilibrium. A further evidence that no permanent defect is induced by the character of the diet is found in the observation that a change to a mixed diet of seeds and vegetables often brought speedy realimentation and recovery.

Despite numerous failures with other proteins than casein, several experiments have already been continued long enough to hold out promise of the possibility of ultimate success. In these the animals were fed on a mixture in which the casein content was gradually decreased and then entirely replaced by vegetable protein.*

RAT 16.—This rat, after a preliminary feeding on dog biscuit-lard diet, was fed through Period 2 with a mixture containing casein 12, excelsin 6, sugar 15, starch 29.5, lard 30, agar 5 and salt mixture I 2.5 per cent. This contained 2.74 per cent of nitrogen. During Period 3 the proportions of excelsin and casein were 9 per cent, the rest of the mixture remaining the same and containing 2.75 per cent of nitrogen. During Period 4 the proportion of excelsin was made 12 and casein 6 per cent, and the diet (otherwise as before) contained 2.8 per cent of nitrogen. Throughout Period 5 the casein was wholly replaced by excelsin, but otherwise the mixture was that above given. The nitrogen content of this diet was 2.94 per cent.

TABLE XXVI.—SUMMARY OF DATA ON RAT 16, FED FOR 210 DAYS ON MIXTURES CONTAINING CASEIN AND EXCELSIN AS THE ONLY PROTEINS.—DAILY AVERAGES.

PERIOD 1, 28 DAYS.—DOG BISCUIT-LARD DIET.

Date of experiment.	Body-weight.	Intake. Food.	Intake. Nitrogen.	Nitrogen output. Urine.	Nitrogen output. Fæces.	Nitrogen output. Total.	N-utilization.	N-balance.
1910.	gm.	gm.	gm.	gm.	gm.	gm.	p. ct.	gm.
Jan. 24....	238.8	......	......	......	......	......	......	......
31....	235.2	8.4	0.132	0.103	0.025	0.128	81	+0.004
Feb. 7....	225.0	6.5	0.106	0.084	0.024	0.108	77	−0.002
14....	226.8	8.5	0.163	0.102	0.029	0.131	82	+0.032
21....	219.5	5.7	0.110	0.088	0.018	0.106	84	+0.004
PERIOD 2, 42 DAYS.—CASEIN 12 PER CENT, EXCELSIN 6 PER CENT.								
1910.								
Feb. 28....	227.8	6.1	0.164	0.141	0.017	0.158	90	+0.006
Mar. 7....	230.0	7.8	0.211	0.150	0.033	0.183	84	+0.028
14....	225.6	7.0	0.189	0.154	0.029	0.183	85	+0.006
21....	226.8	6.6	0.180	0.128	0.018	0.146	90	+0.034
28....	234.0	7.4	0.205	0.156	0.024	0.180	88	+0.025
Apr. 4....	227.8	6.9	0.191	0.148	0.026	0.174	86	+0.017
PERIOD 3, 35 DAYS.—CASEIN 9 PER CENT, EXCELSIN 9 PER CENT.								
1910.								
Apr. 11....	225.5	7.5	0.207	0.160	0.031	0.191	85	+0.016
18....	224.0	7.0	0.193	0.180	0.021	0.201	89	−0.008
25....	229.5	7.6	0.211	0.172	0.023	0.195	89	+0.016
May 2....	234.2	8.5	0.234	0.169	0.027	0.196	88	+0.038
9....	240.0	8.7	0.238	0.175	0.025	0.200	89	+0.038

*For a description of the vegetable proteins used see Osborne; Die Pflanzenproteine, Ergebnisse der Physiologie, 1910, x, p. 47.

TABLE XXVI.—SUMMARY OF DATA ON RAT 16, FED FOR 210 DAYS ON MIXTURES CONTAINING CASEIN AND EXCELSIN AS THE ONLY PROTEINS.—DAILY AVERAGES.—Cont'd.

PERIOD 4, 14 DAYS.—CASEIN 6 PER CENT, EXCELSIN 12 PER CENT.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
May 16....	232.1	7.5	0.210	0.167	0.028	0.195	87	+0.015
23....	233.5	7.2	0.201	0.177	0.020	0.197	90	+0.004
PERIOD 5, 119 DAYS.—EXCELSIN 18 PER CENT.								
1910.								
May 30....	235.6	7.2	0.215	0.165	0.024	0.189	89	+0.024
June 6....	235.0	8.1	0.238	0.192	0.020	0.212	92	+0.026
13....	231.8	7.2	0.211	0.192	0.022	0.214	90	−0.003
20....	228.5	7.3	0.215	0.173	0.026	0.199	88	+0.016
27....	218.9	4.7	0.139	0.141	0.015	0.156	89	−0.017
July 4....	216.2	7.4	0.217	0.150	0.025	0.175	88	+0.042
11....	216.5	6.3	0.186	0.155	0.020	0.175	89	+0.011
18....	220.7	7.6	0.225	0.158	0.025	0.183	89	+0.042
25....	207.5	6.6	0.193	0.181	0.027	0.208	86	−0.015
Aug. 1....	214.5	7.3	0.214	0.159	0.017	0.176	92	+0.038
8....	220.0	7.9	0.231	0.141	0.016	0.157	93	+0.074
15....	212.7	8.2	0.242	0.196	0.025	0.221	90	+0.021
22....	219.4	8.3	0.244	0.170	0.020	0.190	92	+0.054
29....	216.3	8.6	0.252	0.159	0.025	0.184	90	+0.068
Sept. 5....	209.5	8.9	0.262	0.212	0.037	0.249	86	+0.013
12....	197.0	7.9	0.234	0.229	0.022	0.251	91	−0.017
19....	160.3	6.1	0.180	0.223	0.039	0.262	78	−0.082
Dead								

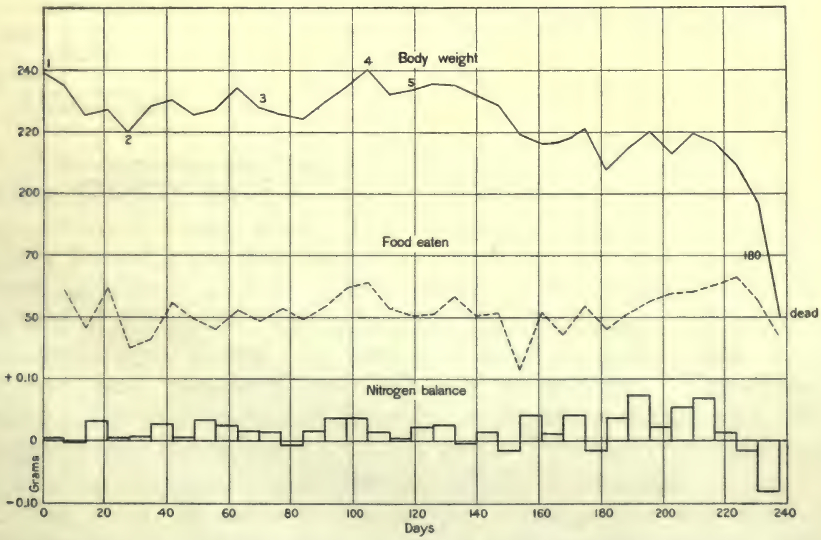

CHART XVII.—Rat 16 fed 210 days on Casein and Excelsin as the only proteins. See p. 44. Numbers on Body-weight line indicate time at which each period began.

RAT 70.—This rat was fed during Period 1 with a mixture containing casein 9, pea legumin 9, sugar 15, starch 29.5, lard 30, agar 5, and salt mixture No. 1, containing 2.5 per cent. This contained 2.75 per cent of nitrogen. During Period 2, the diet contained 18 per cent of legumin as the only protein, the proportion of the other constituents being unchanged. The nitrogen content was 2.97 per cent.

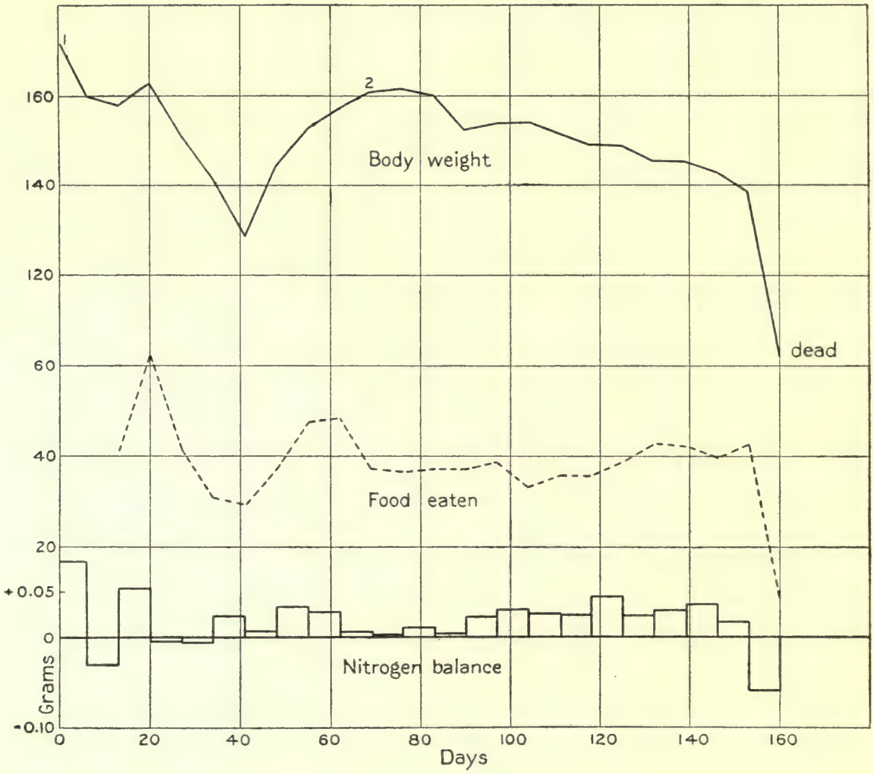

CHART XVIII.—Rat 70, fed 160 days on Casein and Pea-Legumin as the only proteins. See p. 47. Numbers on Body-weight line indicate time at which each period began.

TABLE XXVII.—SUMMARY OF DATA ON RAT 70, FED FOR 160 DAYS ON MIXTURES CONTAINING CASEIN AND PEA LEGUMIN AS THE ONLY PROTEINS.—DAILY AVERAGES.

PERIOD 1, 69 DAYS.—CASEIN 9 PER CENT, PEA LEGUMIN 9 PER CENT.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Apr. 5....	171.7	......	......	......	......	......	......	......
11....	160.0	9.6	0.267	0.159	0.026	0.185	90	+0.082
18....	157.8	5.9	0.164	0.181	0.015	0.196	91	−0.032
25....	162.5	8.9	0.249	0.179	0.016	0.195	94	+0.054
May 2....	151.0	5.9	0.164	0.153	0.016	0.169	90	−0.005
9....	141.2	4.4	0.122	0.118	0.011	0.129	91	−0.007
16....	128.6	4.2	0.115	0.075	0.017	0.092	85	+0.023
23....	144.5	5.3	0.145	0.130	0.009	0.139	94	+0.006
30....	152.8	6.8	0.186	0.131	0.022	0.153	88	+0.033
June 6....	157.8	6.9	0.187	0.139	0.021	0.160	89	+0.027
13....	160.6	5.3	0.143	0.124	0.015	0.139	90	+0.004
PERIOD 2, 91 DAYS.—PEA LEGUMIN 18 PER CENT.								
1910.								
June 20....	161.3	5.2	0.155	0.141	0.013	0.154	92	+0.001
27....	160.0	5.3	0.158	0.128	0.020	0.148	87	+0.010
July 4....	152.3	5.3	0.156	0.134	0.019	0.153	88	+0.003
11....	153.8	5.5	0.163	0.125	0.017	0.142	90	+0.021
18....	154.0	4.7	0.140	0.096	0.014	0.110	90	+0.030
25....	151.5	5.1	0.151	0.107	0.019	0.126	87	+0.025
Aug. 1....	149.0	5.0	0.150	0.111	0.015	0.126	90	+0.024
8....	148.7	5.5	0.163	0.103	0.116	0.119	90	+0.044
15....	145.3	6.1	0.180	0.140	0.018	0.158	90	+0.022
22....	145.5	6.0	0.179	0.133	0.017	0.150	91	+0.029
29....	142.6	5.7	0.168	0.116	0.017	0.133	90	+0.035
Sept. 5....	138.5	6.0	0.176	0.135	0.025	0.160	86	+0.016
12....	102.0	1.2	0.034	0.082	0.013	0.095	62	−0.061
Dead								

RAT 71.—This rat was fed during Period 1 with a mixture containing casein 12, glutenin 6, sugar 15, starch 29.5, lard 30, agar 5, salt mixture I 2.5 per cent. This contained 2.69 per cent nitrogen. During Period 2 the diet contained casein 12, glutenin 6, sugar 15, starch 24.5, lard 35, agar 5, salt mixture I 2.5 per cent, with 2.68 per cent of nitrogen. During Period 3 the diet consisted of glutenin 16.4, sugar 13.6, starch 22.3, lard 40.9, agar 4.5, salt mixture I 2.3 per cent, and contained 2.60 per cent of nitrogen. Throughout Period 4, the diet contained glutenin 18, sugar 15, starch 14.5, lard 45, agar 5 and salt mixture I 2.5 per cent. This diet contained 2.83 per cent of nitrogen which belonged wholly to glutenin.

Rat 71 is still alive at the present writing after 217 days of exclusive diet containing glutenin as its only protein, and 286 days including the casein and glutenin period.

TABLE XXVIII.—SUMMARY OF DATA ON RAT 71, FED FOR 244 DAYS ON MIXTURES CONTAINING CASEIN AND GLUTENIN AS THE ONLY PROTEINS.—DAILY AVERAGES.

PERIOD 1, 13 DAYS.—CASEIN 12 PER CENT, GLUTENIN 6 PER CENT.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.*
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Apr. 5....	257.5	......	......	......	......	......	......	......
18....	210.5	6.8	0.184	0.183	0.030	0.213	84	−0.029

PERIOD 2, 56 DAYS.—CASEIN 12 PER CENT, GLUTENIN 6 PER CENT.

Date of experiment.	Body-weight.	Food.	Nitrogen.	Urine.	Fæces.	Total.	N-utilization.	N-balance.*
1910.								
Apr. 25....	238.7	11.0	0.297	0.249	0.031	0.280	90	+0.017
May 2....	225.5	4.6	0.124	0.187	0.013	0.200	90	−0.076
9....	222.2	9.6	0.259	0.163	0.017	0.180	93	+0.079
16....	227.1	6.1	0.166	0.130	0.014	0.144	92	+0.022
23....	234.5	7.3	0.196	0.139	0.020	0.159	90	+0.037
30....	242.3	8.2	0.218	0.152	0.025	0.177	89	+0.041
June 6....	256.1	10.9	0.290	0.196	0.040	0.236	86	+0.054
13....	255.0	6.5	0.173	0.169	0.033	0.202	81	−0.029

PERIOD 3, 35 DAYS.—GLUTENIN 16.4 PER CENT.

Date of experiment.	Body-weight.	Food.	Nitrogen.	Urine.	Fæces.	Total.	N-utilization.	N-balance.*
1910.								
June 20....	248.5	6.0	0.155	0.134	0.026	0.160	83	−0.005
27....	252.6	7.4	0.190	0.133	0.035	0.168	82	+0.022
July 4....	238.1	4.5	0.115	0.105	0.016	0.121	86	−0.006
11....	240.5	5.9	0.153	0.091	0.029	0.120	81	+0.033
18....	236.5	5.0	0.132	0.087	0.017	0.104	87	+0.028

PERIOD 4, 182 DAYS.—GLUTENIN 18 PER CENT.

Date of experiment.	Body-weight.	Food.	Nitrogen.	Urine.	Fæces.	Total.	N-utilization.	N-balance.*
1910.								
July 25....	241.0	6.6	0.174	0.129	0.022	0.151	87	+0.023
Aug. 1....	261.8	8.5	0.240	0.140	0.021	0.161	91	+0.079
8....	259.2	8.0	0.226	0.138	0.030	0.168	87	+0.058
15....	255.1	6.8	0.194	0.135	0.017	0.152	91	+0.042
22....	247.5	7.0	0.195	0.107	0.020	0.127	90	+0.068
29....	245.0	6.7	0.190	0.075	0.016	0.091	92	+0.099
Sept. 5....	241.5	7.5	0.212	0.132	0.030	0.162	86	+0.050
12....	235.2	6.3	0.179	0.171	0.014	0.185	92	−0.006
19....	244.8	9.3	0.263	0.209	0.040	0.249	85	+0.014
26....	240.0	8.3	0.237	0.191	0.019	0.210	92	+0.027
Oct. 3....	238.5	7.7	0.218	0.186	0.019	0.205	91	+0.013
10....	239.2	6.7	0.191	0.151	0.028	0.179	85	+0.012
17....	228.0	6.5	0.186	0.163	0.023	0.186	88	0.000
24....	223.5	6.9	0.198	0.162	0.037	0.199	81	−0.001
31....	238.7	9.4	0.269	0.202	0.037	0.239	86	+0.030
Nov. 7....	253.4	9.8	0.280	0.188	0.038	0.226	86	+0.054
14....	248.0	7.6	0.217	0.176	0.033	0.209	85	+0.008
21....	250.8	7.8	0.221	0.158	0.045	0.203	80	+0.018
28....	254.0	7.4	0.211	0.144	0.037	0.181	82	+0.030
Dec. 5....	253.6	7.9	0.226	0.174	0.039	0.213	83	+0.013
12....	254.0	9.0	0.252	0.208	0.040	0.248	84	+0.004
19....	262.5	11.0	0.301	0.214	0.064	0.278	79	+0.023
26...........		10.8	0.296	0.220	0.052	0.272	82	+0.024
1911.								
Jan. 2....	279.8	11.7	0.321	0.222	0.049	0.271	85	+0.050
9....	273.5	8.7	0.237	0.208	0.035	0.243	85	−0.006
16....	275.5	11.5	0.317	0.270	0.038	0.308	88	+0.009

*In respect to the relatively high nitrogen balance compare last foot-note on page 7.

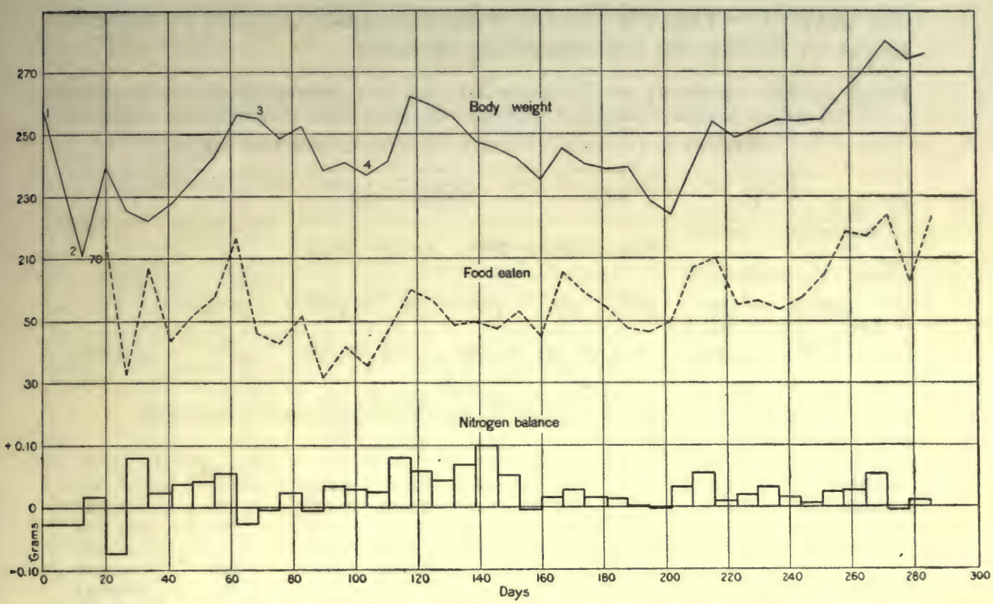

CHART XIX.—Rat 71 fed 286 days on Casein and Glutenin as the only proteins. See p. 48. Numbers on Body-weight line indicate time at which each period began.

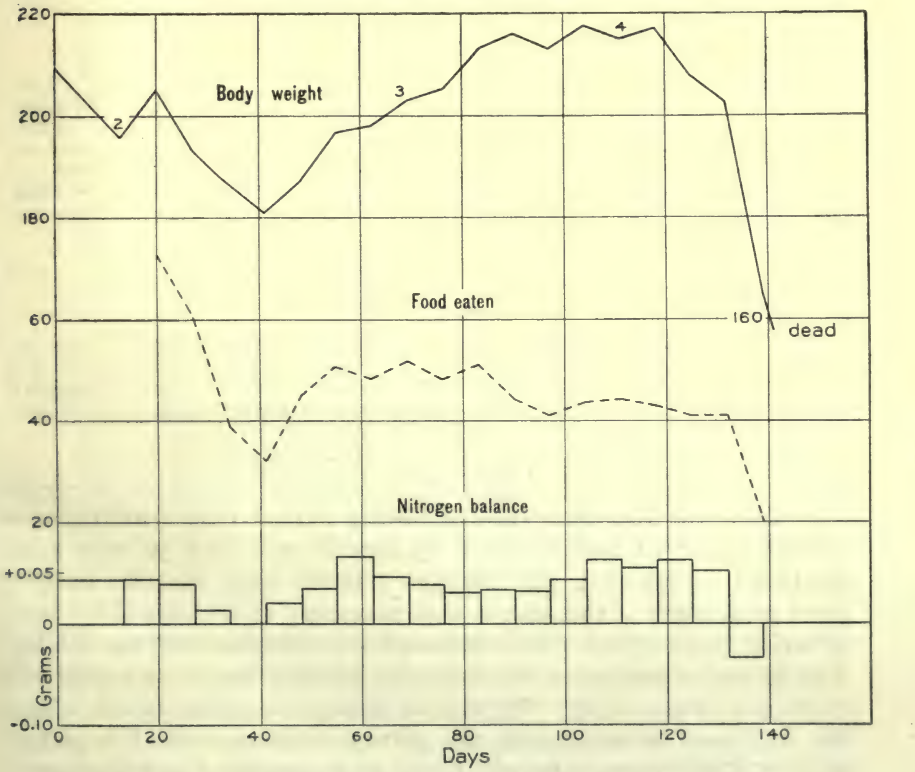

CHART XX.—Rat 72 fed 141 days on Casein and Glutenin as the only proteins. Numbers on Body-weight line indicate time at which each period began.

RAT 72.—This rat was fed with the same mixtures as those fed to rat 71 during the corresponding periods.

TABLE XXIX.—SUMMARY OF DATA ON RAT 72, FED FOR 141 DAYS ON MIXTURES CONTAINING CASEIN AND GLUTENIN AS THE ONLY PROTEINS.—DAILY AVERAGES.

PERIOD 1, 13 DAYS. CASEIN 12, GLUTENIN 6 PER CENT.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Apr. 5....	209.5	.....	.....	.....	.....	.....	.....	.....
18....	195.7	8.7	0.233	0.145	0.045	0.190	81	+0.043
PERIOD 2, 56 DAYS.—CASEIN 12 PER CENT, GLUTENIN 6 PER CENT.								
1910.								
Apr. 25....	205.0	10.4	0.281	0.186	0.053	0.239	81	+0.042
May 2....	193.3	8.7	0.235	0.147	0.049	0.196	79	+0.039
9....	186.4	5.5	0.150	0.126	0.012	0.138	92	+0.012
16....	181.0	4.6	0.123	0.114	0.009	0.123	93	0.000
23....	187.2	6.4	0.170	0.126	0.024	0.150	86	+0.020
30....	196.4	7.2	0.192	0.120	0.038	0.158	80	+0.034
June 6....	198.0	6.9	0.214	0.123	0.025	0.148	88	+0.066
13....	202.8	7.4	0.196	0.131	0.019	0.150	90	+0.046
PERIOD 3, 42 DAYS.—GLUTENIN 16.4 PER CENT.								
1910.								
June 20....	205.0	6.9	0.176	0.110	0.028	0.138	84	+0.038
27....	213.0	7.3	0.187	0.112	0.045	0.157	76	+0.030
Ju.y 4....	216.1	6.3	0.162	0.097	0.032	0.129	80	+0.033
11....	213.0	5.8	0.152	0.092	0.029	0.121	81	+0.031
18....	217.7	6.2	0.164	0.085	0.035	0.120	79	+0.044
25....	214.9	6.3	0.166	0.072	0.032	0.104	81	+0.062
PERIOD 4, 30 DAYS.—GLUTENIN 18 PER CENT.								
1910.								
Aug. 1....	217.3	6.1	0.170	0.090	0.025	0.115	85	+0.055
8....	207.7	5.8	0.165	0.078	0.025	0.103	85	+0.062
15....	202.5	5.9	0.166	0.099	0.016	0.115	90	+0.051
22....	165.0	2.9	0.081	0.079	0.037	0.116	54	−0.035
24....	157.5							
Dead								

RAT 73.—This rat was fed during Period 1 on a mixture containing casein 12, zein 6, sugar 15, starch 29.5, lard 30, agar 5, salt mixture I 2.5 per cent, and nitrogen 2.38 per cent. In order to secure good utilization of the zein it was necessary to hydrate it by incorporating in the food 10 cc. of water per 100 grams of the mixture. The nitrogen content of the different batches therefore varied from 2.30 to 2.48 per cent. The actual nitrogen content of each batch fed was used in calculating the nitrogen balance which is given in table XXX. During Period 2, the diet contained pea legumin 18,

sugar 15, starch 29.5, lard 30, agar 5, salt mixture I 2.5, and nitrogen 2.97 per cent.

TABLE XXX.—SUMMARY OF DATA ON RAT 73, FED FOR 181 DAYS ON A MIXTURE CONTAINING CASEIN AND ZEIN AS THE ONLY PROTEINS AND FOR 53 DAYS ON ONE CONTAINING PEA LEGUMIN AS THE SOLE PROTEIN.—DAILY AVERAGES.

PERIOD 1, 181 DAYS. CASEIN 12 PER CENT, ZEIN 6 PER CENT.

Date of experiment.	Body-weight.	Intake.		Nitrogen output.			N-utilization.	N-balance.
		Food.	Nitrogen.	Urine.	Fæces.	Total.		
1910.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*p. ct.*	*gm.*
Apr. 5....	179.0	......	......	......	......	......	......	......
11....	174.0	3.9	0.092	0.177	0.024	0.201	74	−0.109
18....	165.0	7.5	0.177	0.171	0.018	0.189	90	−0.012
25....	167.0	8.0	0.189	0.163	0.013	0.176	93	+0.013
May 2....	156.0	5.9	0.143	0.147	0.013	0.160	91	−0.017
9....	140.0	4.0	0.098	0.123	0.008	0.131	92	−0.033
16....	127.8	3.2	0.080	0.107	0.009	0.116	89	−0.036
23....	137.4	5.2	0.123	0.111	0.006	0.117	95	+0.006
30....	126.1	3.1	0.072	0.084	0.007	0.091	90	−0.019
June 6....	126.5	4.4	0.102	0.108	0.005	0.113	95	−0.011
13....	129.5	4.8	0.112	0.091	0.008	0.099	93	+0.013
20....	128.2	4.0	0.094	0.084	0.006	0.090	94	+0.004
27....	128.7	3.7	0.086	0.075	0.004	0.079	95	+0.007
July 4....	123.3	3.4	0.080	0.081	0.006	0.087	92	−0.007
11....	123.3	3.9	0.093	0.080	0.007	0.087	92	+0.006
18....	125.0	3.7	0.088	0.064	0.007	0.071	92	+0.017
25....	118.3	3.6	0.084	0.076	0.005	0.081	94	+0.003
Aug. 1....	119.8	4.1	0.094	0.063	0.006	0.069	94	+0.025
8....	124.9	4.9	0.112	0.075	0.005	0.080	96	+0.032
15....	127.5	5.0	0.133	0.092	0.009	0.101	93	+0.032
22....	132.3	2.8	0.070	0.105	0.012	0.117	83	−0.047
29....	135.2	8.1	0.200	0.082	0.011	0.093	94	+0.107
Sept. 5....	131.5	5.4	0.134	0.096	0.011	0.107	92	+0.027
12....	132.5	5.1	0.126	0.110	0.007	0.117	94	+0.009
19....	138.2	6.1	0.151	0.128	0.014	0.142	91	+0.009
26....	142.8	7.1	0.170	0.139	0.017	0.156	90	+0.014
Oct. 3....	143.6	7.1	0.167	0.144	0.015	0.159	91	+0.008
PERIOD 2, 53 DAYS.—PEA LEGUMIN 18 PER CENT.								
1910.								
Oct. 10....	136.3	5.2	0.153	0.168	0.036	0.204	76	−0.051
17....	127.5	5.1	0.150	0.165	0.020	0.185	87	−0.035
24....	121.1	4.6	0.136	0.142	0.018	0.160	87	−0.024
31....	110.7	4.3	0.129	0.133	0.023	0.156	82	−0.027
Nov. 7....	107.2	4.6	0.135	0.132	0.019	0.151	86	−0.016
14....	99.0	3.9	0.115	0.121	0.012	0.133	90	−0.018
21....	97.5	4.1	0.121	0.118	0.011	0.129	91	−0.008
29....	70.0							

These observations have thus shown the sufficiency of the artificial dietaries to maintain full-grown small animals for long periods of time (from 50 to 286 days) in nutritive equilibrium. In many experiments, such as Nos. 11, 14, 16, and 72, here reported, the animals died rather suddenly, without any previous period of notable

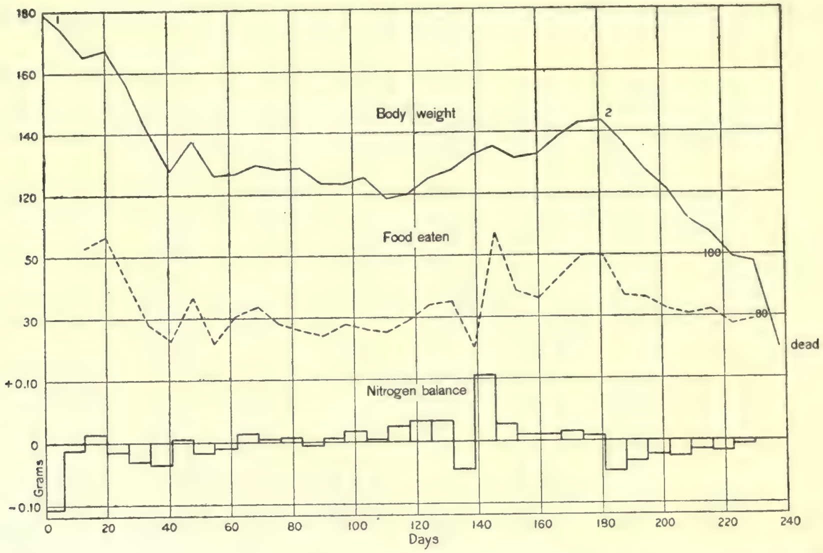

CHART XXI.—Rat 73 fed 181 days on Casein and Zein, and for 53 days on Pea-Legumin as the only proteins. See p. 50. Numbers on Body-weight line indicate time at which each period began.

decline sufficient to explain their ultimate death. This fact strongly suggests that death was not necessarily attributable to any primary nutritive defect, but rather to incidental causes. Occasional fatalities will not surprise those who have experienced the difficulty of protecting a large number of rats, under the conditions noted, from the appearance of infectious or parasitic maladies which may become fatal. In the same surroundings sudden death has also come to not a few of our animals on ordinary mixed diets consisting of seeds and vegetables. In some cases obvious causes were revealed at autopsy; but systematic post-mortem examinations have not been attempted.

With young rats fed similarly we have succeeded in maintaining weight, although with little if any growth. In our preliminary studies of growing animals the food intake was not determined with appropriate care to correlate our findings with the altered curves of growth; hence the insufficient diet rather than any chemical deficiency may have been a possible cause of arrested development. Rats of 30 grams initial weight have been kept by us for many days without gaining weight when fed with a mixture containing a single protein; with desiccated milk in the food they subsequently attained a perfectly normal growth.

Despite the obstacles encountered we are inspired to the belief that with modifications in the feeding suggested by our first year's experiments still further progress can be made. Meanwhile further conclusions respecting the inadequacy of the individual proteins for nutritive functions are not justified.

SUMMARY.

The problems of nutrition have been reviewed in this paper in the light of the newer knowledge of the chemical structure of the proteins. The possibilities of protein synthesis in animals and the conditions which this postulates; the significance of the availibility, palatability, and physical texture of the food-intake; the suggested role of various accessories—inorganic salts, lipoids, etc.; the distinction between the nutritive demands during the period of growth and those of later adult life, are brought within the range of discussion. The literature on experiments in which isolated food-substances have been fed to animals is discussed in some detail, with a critical consideration of some of the essential conditions of investigation which are demanded in successful research in this direction.

The methods of metabolism study with white rats used in this research are described and illustrated. Control feeding trials showed that the animals can be maintained in nutritive equilibrium and health for periods of many months under the conditions of experiment adopted. The failure to eat sufficient food is indicated as a cause for the unsuccessful termination of numerous experiments. The facts presented exclude the probability that monotony of diet is an insurmountable obstacle to nutritive success.

Numerous experiments are reported in which casein formed the sole nitrogenous constituent of the dietary. In this connection it is shown that the make-up of the inorganic constituents of the diet exercises an influential effect on the nutritive efficiency of the dietary. From the experience thus gained a "basal" ration was constructed on which rats were kept many months in good health. Some of these experiments in which the animals exhibited no noteworthy alterations in weight and showed a good gain in nitrogen are, as far as the authors are aware, the most successful recorded attempts at artificial nutrition with a constant mixture of pure food-stuffs, containing only a single protein. Satisfactory experience also followed the gradual complete substitution of the casein by other proteins, one animal continuing more than 217 days on a diet in which the sole protein was glutenin.

With young rats it has been possible to maintain weight with dietaries like those just mentioned, although with little if any growth. The limitations of the method are discussed and plans for continued investigation indicated.

JANUARY 1911.

FEEDING EXPERIMENTS WITH ISOLATED FOOD-SUBSTANCES.

BY

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FEEDING EXPERIMENTS WITH ISOLATED FOOD-SUBSTANCES.

PART II.

INTRODUCTION.

In Publication 156 of the Carnegie Institution of Washington* we have discussed some of the problems of nutrition which have been raised by the newer investigations in the field of protein chemistry. The literature bearing on the feeding of isolated proteins was there reviewed in some detail, together with critical considerations of previously available experimental data. We described a plan for the study of metabolism and illustrated a method of investigation in which white rats were the experimental animals. For the details involved, our earlier paper must be consulted. A few protocols were there presented to show that the outlined mode of investigation offered a promising means for attacking certain questions in the field of nutrition.

INFLUENCE OF VARIOUS CONDITIONS ON NUTRITION OF WHITE RATS.

Numerous contingencies may arise to modify or vitiate the results of experiments in which animals are kept in cages and fed upon artificially prepared mixtures of isolated food-stuffs, quite independent of the factors inherent in the food-stuffs themselves or the combinations in which they are exhibited. Among these possibilities, the *caging* itself, involving continued restraint and limited opportunity for exercise, suggests an unfavorable environment. This factor can at length be disposed of.

Donaldson has concluded, from the best data obtainable, that "the three-year-old white rat is very old, and is justly comparable to a man of 90 years."† Rats have been kept in our cages in apparent good health and without difficulty during periods of more than 14 months—a very considerable part of the span of life in these animals (cf. Charts XXIII, XXIX, XXX).

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*Feeding experiments with isolated food-substances, by Thomas B. Osborne and Lafayette B. Mendel, with the co-operation of Edna L. Ferry. 1911. Pp. 53.

†H. H. Donaldson: A comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume, New York, 1906, p. 6.

Monotony of diet has been urged as an obstacle to success where the same food mixtures are daily furnished without change over long periods of time. Very closely associated with this is the question of the *palatability* of the diet. The two factors need, however, to be distinguished. The palatability of the diet has, perhaps, been overemphasized in recent years in its bearing on the real nutritive value of foods. It applies primarily to the individual with highly organized nervous system and psychical functions. The quality found in foods which are unpalatable because they disgust or nauseate is something positive; the negative property of lack of palatability, *i. e.*, absence of stimulating taste, etc., is not necessarily a serious obstacle. In any event the palatability of the diet is difficult to determine or regulate and in attempting to control it experimentally in animals physiologists have been guided very largely by anthropomorphistic considerations.

We have now gathered observations which lead us to dismiss the idea that monotony *per se* leads to anorexia or other forms of nutritive failure in our animals, despite the comment which this feature has received from other investigators. There is no convincing reason why a continued unvaried diet should necessarily be unphysiological; one need only recall the fact that the diet of all sucklings is the same from day to day, and that many of the domestic animals are satisfactorily maintained on rations which are scarcely altered in qualitative make-up except at long intervals. We have observed rats in the same cage for considerably more than a year, during which the daily diet was invariably furnished in the form of our food-pastes. In some of these the composition of the paste was practically the *same* during these very long periods (cf. Charts XXVII, XXVIII, XXIX, XXX). It is true that we could point to many failures to maintain rats on an unchanged diet continued over much shorter periods. One must not, however, here confuse monotony with the real cause of decline. In these latter cases some deficiency or defect in the monotonous feeding sooner or later brings on a physiological state where anorexia occurs; and the advantage which a change of diet initiates ought primarily to be ascribed to the alteration in the food ingredients rather than the relief from the sameness of the intake.

Among factors referring more directly to the nature of the food itself, the *physical texture* and *digestibility* of the nutrients must be taken into consideration. The structure of the food materials may, under ordinary conditions of diet, influence its utilization in no small degree; and the low "coefficients of digestibility" shown by many foods of plant origin testify to this fact. In our experiments the products fed were isolated and reduced to a state of very fine comminution. At most, therefore, some inherent indigestibility of the individual foodstuffs employed might be concerned. Experiments

by M. S. Fine,* while they do not completely do away with this possibility, make it more evident than before that incomplete digestion is, in the case of plant products, for the most part associated with the peculiar vegetable tissues therein contained, rather than a specific resistance of the isolated nutrients.

The need of "*roughage*" to facilitate the normal evacuation of the gut has also been debated. We have, as a general procedure, added the indigestible polysaccharide carbohydrate agar-agar to food-pastes in order to approximate more nearly the conditions which prevail where cellulose enters into the mixed dietary. It can not be maintained, however, that this is necessary for satisfactory nutrition; for we have maintained animals over a year on foods (cf. Chart XXIX) devoid of indigestible principles, if perhaps an exception be made of some of the inorganic ingredients. It is well known that inorganic salts, notably bone ash, may exert the same influence as cellulose in giving bulk to the fæces; and they are often so employed in the technique of metabolism experiments at the present time.†

Aside from the proteins, in which our experimental interest has been primarily centered, our attention has been drawn more and more to those components of the diet which are not sources of energy, yet fundamentally indispensable—namely, the *inorganic compounds.* It is possible that further investigation will compel the inclusion of some of the more vaguely defined and unknown members of the groups spoken of as extractives, lipoids, etc., in this category. Every attempt made by us to approach the solution of the problem of inorganic salts in the dietary has brought fresh surprises.

When Forster‡ fed dogs and pigeons on salt-free foods he made the interesting observation that the animals speedily died—more rapidly even than when all food was withheld. He concluded:

> Der im Uebrigen in Stickstoffgleichgewicht sich befindende thierische Organismus bedarf zu seiner Erhaltung der Zufuhr gewissen Salze; sinkt die Zufuhr unter einer gewisse Grenze oder wird sie gänzlich aufgehoben, so gibt der Körper Salze ab und geht daran zu Grunde.

The classic experiments of Lunin§ on mice led to a somewhat different interpretation of the need of salts. He showed that the animals survived longer on a diet containing an addition of sodium carbonate to the ash-free food than when sodium chloride was added. In the latter case the duration of life corresponded approximately with that observed on a salt-free dietary. From these facts it was argued that the foremost value of the sodium lies in its capacity to neutralize the acids (sulphuric, phosphoric) formed in the metabolism

*M. S. Fine: Dissertation, Yale University, 1911 (unpublished). Cf. Mendel and Fine: Journal of Biological Chemistry, 1911, vols. x and xi.

†Cf. Lothrop: American Journal of Physiology, 1909, xxiv, p. 297.

‡Forster: Zeitschrift für Biologie, 1873, ix, pp. 297–380.

§Lunin: Zeitschrift für physiologische Chemie, 1881, v, p. 31.

of proteins. Sodium chloride obviously has no potential neutralizing power. If the usefulness of the salts were associated solely with their specific character as salts, the salts of sodium ought to be somewhat comparably efficient.

The *function of the inorganic salts* is by no means exhausted, however, by the simple action of chemical equilibrium. It would lead us too far afield in this place to discuss the problem in detail. Charts XI, XII, and XIII, Part I, pp. 38–39) showing the marked differences induced by alterations in the inorganic salts of the diet, the other food components remaining unchanged, are highly suggestive. We have since then made numerous attempts to improve upon the salt mixture empirically selected and prepared somewhat in imitation of the ash of milk. Rats were kept alive (while they steadily declined) 84 days on a food mixture which analysis showed to contain only minimal, inevitable traces of ash (0.16 per cent, a considerable part of which was phosphoric acid derived from the casein). Chlorides were entirely lacking, distilled water being furnished for drinking. In view of this it is necessary to proceed with extreme caution in drawing conclusions from observations extending over brief periods. We shall refer to the subject again, it being sufficient here to emphasize the subtle and specific value of the salts. The lack of knowledge in this field has furnished an obstacle which we have only lately succeeded in overcoming in part.

Even when all these varied conditions are taken into account, there still remain, as we have pointed out before, *extraneous incidents* and *accidental factors* apart from nutrition itself, which may complicate or vitiate experiments like those projected. Disease, old age, injury, may be mentioned in illustration. Failures to maintain nutrition successfully under such extreme conditions do not necessarily imply a deficiency or inadequacy of the dietary. Accordingly, successful experiments must be given far greater weight than failures, where so many possibilities of detrimental influences, aside from the diet itself, are liable to arise over prolonged periods of observation. Some of the uncertainties have been eliminated by the experience previously gained. For example, the intercurrent diseases of our animals have been almost entirely excluded by the use of rats raised in the laboratory for this research. By the prompt elimination of diseased animals, by scrupulous attention to the conditions of the cages and feeding arrangements—in other words, by painstaking attention to hygienic factors—we have succeeded in maintaining a large number of animals in exceptionally good health, so that they have become the more suitable to permit of accurate conclusions regarding the effects of the diets studied. Furthermore, the age and hereditary factors in our animals are now known to us, so that another source of uncertainty has disappeared.

EARLIER EXPERIENCES OF THE AUTHORS.

As the result of the first year's experiments, it was found possible to maintain rats in health and apparent nutritive equilibrium over considerable periods of time on a mixture of isolated food-substances containing isolated proteins as the source of nitrogenous intake. For example, one protocol (Chart XXX) shows that a full-grown rat* was maintained satisfactorily in this way for more than 217 days on glutenin, the animal continuing on this régime at the time when the earlier report was prepared for publication. Rats were likewise maintained on diets in which other proteins, notably casein alone or in combination with isolated vegetable proteins, formed the sole nitrogenous food component, over periods of time exceeding any previously reported, at least under conditions in which the "purity" of the dietary substances was carefully maintained unchanged over equally long periods of time. By maintenance we do not merely mean that the animals remain alive. No feeding experiment is to be regarded as successful in fulfilling the nutritive requirements unless the animals approximately maintain their weight and health (or make normal growth if they are at a stage where this is still to be expected).

Although these apparently successful experiments indicated that the combinations of isolated food-stuffs employed satisfied the nutritive requirements of the rats and consequently constituted a complete food for the maintenance of mature animals, a prolongation of the observations has led to a less favorable outcome. A continuation of the experiments over longer periods has shown that in every case, sooner or later, the animal declined; and unless a change in the diet was now instituted within a comparatively short time the animals died. The Charts XIV, XV, XVI in our earlier paper illustrate this very well. The rats 23, 24, 25 were maintained without noteworthy alterations in weight over 130 to 160 days on a constant mixture including a single protein. The animals ate well, as the food records show, until the final period of decline.

These records can be duplicated, especially in respect to the decline, by many others, as for example Charts XLI, XLII, LXXVIII, LXXIX, LXXX, CII, CXV, CXVI appended to this report. The history of rat 71 is particularly instructive on this point.† This animal (see Chart XXX), weighing 257 grams on April 5, 1910, was put upon a diet containing casein (12 per cent) and glutenin (6 per cent) as the only proteins. Subsequently glutenin alone (16.4 per cent after 69 days and 18 per cent after 104 days) formed the protein of the diet. The rat continued in excellent nutritive

*The earlier data regarding this animal, rat 71, are given in Publication No. 156, Carnegie Institution of Washington, p. 47 ff.

†The earlier data will be found in Publication No. 156, Carnegie Institution of Washington, pp. 47–48.

condition, eating well and exhibiting favorable nitrogen balances, until the end of $9\frac{1}{2}$ months, when a gradual decline was observed. When the animal, at the end of a total feeding period of 335 days (42 days after the onset of the decline) was reduced to 162.5 grams in weight and near death, an attempt was made to see whether the decline was due solely to improper food or to the onset of old age or disease. With mixed food realimentation took place at once and the rat regained its weight in a week. A resumption of the former glutenin food during 35 days gradually led to a second decline, which was promptly checked by a change in the diet involving only the non-protein components of the food mixture. Here, then, is a record of the feeding of a full-grown rat, with the exception of 7 days, during a period of 454 days on a diet of isolated food-stuffs and on a diet containing a single protein, glutenin, for 371 days. This observation is remarkable because of the exceptional duration of the experiment. It is apparent, therefore, that *as a maintenance diet our food lacked something other than protein and energy.*

It remains to be shown precisely what the lacking component of our earlier diets is, whether some organic constituent or a peculiar proportion of inorganic ingredients. In any event it is evident that our original artificial food mixtures are incapable of supporting life indefinitely. Aside from this, however, records like that of rat 71 living on glutenin as the sole source of protein (see Chart XXX), or rat 133 (Chart LXX) on edestin, in contrast with rats XI, XIV, 146, and 157 (Charts CXXVI, CXXVII, CXXVIII, and CXXIX) on zein indicate the possibility of nutritive inequalities among the proteins themselves. Marked deficiencies tend to manifest themselves in comparatively short periods of time. In all of these cases the food actually consumed supplied sufficient *energy* for the immediate needs of the rats under investigation.

In the continuation of our experiments we have tried to profit by the first year's experiences. The methods have not been materially altered, except that the determination of the *nitrogen balance* has been omitted for the present. We learned from very numerous trials that it runs parallel with gain or loss of weight, and that the food intake varies closely with the weight of the animal, thereby making a record of the nitrogen unnecessary for judging the nutritive status of the rats employed. The same cages as heretofore have continued to prove very satisfactory. Instead of being rested on glass funnels for the collection of urine, they are now placed over a frequently changed sheet of absorbent paper (paper napkin) upon an enameled tray or pan. The fluid excreta thus promptly absorbed are frequently removed. It has already been pointed out that the food mixtures, prepared in paste form to prevent scattering by the animals and make it possible to obtain accurate records of the quantities eaten,

are not ideal in composition. The inclusion of 20 to 45 per cent of fat in the diet—a condition necessitated by the requirements of the experiments as outlined—seems like an excessive amount; nevertheless the utilization appears to be satisfactory and attempts to devise less objectionable modes of feeding have been unsuccessful in our hands.

ALIMENTARY BACTERIA AND NUTRITION.

In the course of our later studies we have been forced to take cognizance of the possible rôle of the bacterial flora of the alimentary tract in relation to appropriate nutrition. The water-free, fat-rich food characteristic of our experimental dietaries is not, broadly speaking, a particularly favorable medium for the development of certain groups of bacteria. The food of our animals therefore probably introduces into the digestive tube of the experimental animals bacterial invaders somewhat different from those which normally inhabit the alimentary tract of rats living on a free mixed diet. It is quite conceivable, therefore, that the bacterial conditions may be altered markedly as a result of the restriction in the growth of certain groups or the facilitation of the development of still others in the alimentary tract under these changed and sustained conditions of altered diet.* It is well known, for example, that in higher animals the preponderance of acid-producing organisms—to use a single illustration—may lead to an inhibition of the growth of the putrefactive group.

Guided by such considerations and the observation that those rats that have been maintained for long periods on diets with isolated food-stuffs become koprophagists, we have initiated the plan of feeding small quantities of the fæces of rats living on ordinary mixed food to some of our experimental animals, particularly in cases where symptoms of nutritive decline had become manifest. In nearly every instance the occasional addition of a small amount of the fæces from a normally fed rat at once stopped the decline in weight of the experimental animals to which a single protein was being fed. The results in almost all of these cases have been sufficiently striking to warrant a further pursuit of this topic. In our experiments there appears to be an unmistakable favorable influence induced by the occasional addition to the dietary of normal fæces with their high bacterial content. It must not be overlooked that other components besides bacteria, notably inorganic salts and unknown compounds, are also furnished by this means; but the quantities involved have always been very small. Further investigation will be necessary and is already projected.

The procedure in the case of these fæces-feeding trials consisted in introducing small amounts (about 0.5 gm.) of air-dry excrement

*Cf. Herter and Kendall: Journal of Biological Chemistry, 1910, VII, p. 203; Kendall: Journal of the American Medical Association, April 15, 1911.

of rats on mixed food into the cages twice a week. It is an interesting observation that when the rats kept on a mixture of isolated food-substances were offered a choice between their own fæces and those of rats on mixed diets, they invariably chose the fæces of the latter. In many cases we have noticed a marked improvement in the nutritive conditions of animals maintained on a single-protein dietary when other rats were introduced into their cages for breeding purposes. In view of the favorable influence exerted by feeding the fæces of rats living on mixed food, it is quite likely that the presence of the strangers in the cages furnished a suitable opportunity to obtain "normal" fæces. This may explain the favorable results noted, in contrast with the negative effects seen where several rats living on the same single-protein diet have been maintained in the same cage.

The extent of the influence exerted by what we have, in the absence of a better explanation, assumed to be bacterial influences, is illustrated in some of the appended charts, the periods at which the fæces feeding was begun being indicated. The favorable effects have not been confined to experiments with one protein, but are manifested with casein (see Charts XXXIX, XL, XLI, and XLII), with edestin (see Charts LXVI, LXVII, LXVIII, and LXIX), and with gliadin (see Charts CI, CII, and CIII). Two failures may likewise be recorded, viz, an ultimate one with casein (Chart XLI) and a complete one with edestin (Chart LXXVII) as the protein component. These were not due to incapacity of the animals to grow, since further alteration of diet brought marked improvement.

The influence of fæces feeding is especially striking in the case of the gliadin tests, since without the addition of the fæces it has been almost impossible to attain satisfactory nutritive condition with this protein plus the special non-protein components of the food here employed. It is instructive therefore to compare such failures (cf. Period 2, Charts CXV and CXVI) with Charts CI and CIII, in which fæces feeding was resorted to.

In four of the experiments with edestin-food alluded to and recorded on Charts LXVI, LXVII, LXVIII, and LXIX, fresh fæces were not actually introduced into the cages; but the improvement, and even growth, in these young rats is coincident with the opportunity afforded to obtain "normal" fæces when other rats were daily introduced into the cages for a few hours.

In Chart CII is seen the result of an attempt to determine whether the favorable influence of the fæces is actually of bacterial nature. Fæces were fed as in the comparable gliadin experiments (Charts CI and CIII); but they were previously sterilized by thrice repeated heating in an atmosphere of steam. The decline of the animal was not prevented to the same extent with sterilized as with normal fæces. Further trials are necessary in this direction; and our

experience, though limited, invites attention anew to the possible nutritive functions of bacteria in the alimentary tract. Some of the aspects of this problem are referred to in our earlier paper.*

NUTRITION AND GROWTH.

The criteria of adequate nutrition are quite different in the case of *growing* animals from those applying to adults of the same species. During the period of adolescence it is not sufficient to maintain a condition of nutritive equilibrium and constancy of form or body-weight. In this stage of an animal's existence there should be evidences of development, and *growth* should manifest itself in a change of size. The curve of growth, expressed in changes of body-weight, is remarkably constant and characteristic for each species under the ordinary conditions of nutrition and environment. The individual values may at times fluctuate about a mean; but in the majority of cases the excursions from the average are not extensive.

In Chart XXII are reproduced curves illustrating the average normal rate of growth of the white rat, both male and female. The statistics for two of the curves are taken from Donaldson,† whose observations we have repeatedly verified in their general features. A third curve on the same chart represents the results of our own observations on the growth of the female white rat, regarding which data are less abundant. It will be noted that the curves of growth for the two sexes do not completely coincide in type. After an age of 70 days, represented by a body-weight of about 100 grams, the rate of growth is somewhat slower in the female than in the male. Indeed, the females rarely attain the large weight and size exhibited by the normal adult males of the same age, even in the case of animals from the same litter. We gain the impression that our "breed" of rats may in general be somewhat smaller than those measured by Donaldson and his collaborators. At any rate, the data available for statistical purposes are not very extensive and the curves here presented must have only a provisional value until more numerous measurements are made. In connection with certain of our experiments it may be stated that "the effect of mating on the growth-curve for the males can probably be neglected."‡ In the case of females, the effect of the bearing of young is, according to Watson,§ "to render the mated rats slightly heavier than the unmated—some of the excessive weight being due to the larger amount of fat present in the mated animals." Two charts (XXIV, XXV) are appended

*Carnegie Institution of Washington, Publication No. 156, p. 3.

†Donaldson: A comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume, New York, 1906.

‡Cf. Donaldson: *ibid*, p. 8.

§Watson: Journal of Comparative Neurology, 1905, xv, p. 523.

to illustrate the influence of the course of pregnancy on the growth-curve of female rats of different sizes.

Making allowance for these minor divergencies, the striking uniformity in the progress of development in an animal nevertheless is a specific racial characteristic, and gives to the *curve of growth* a unique value as an index of the conditions which determine it. Growth is affected by two factors: nutrition, and what Rubner has termed "Wachstumstrieb" or growth-impulse. The latter factor is inherent in the animal. The limits are determined by heredity and can not be altered materially by the most abundant diet. "Eine noch so reichliche Ernährung vermag die in der Rasse und deren Vererbung gelegenen Grössen- und Massenbegrenzungen nicht zu mehren."*

We are not prepared, at this time, to discuss the nature of the hereditary factor or impelling "force" in growth.† Aron writes:

> Die Natur des Wachstumstriebes ist dunkel. Sie ist eine Funktion der Zellen, im besonderen der jugendlichen Zellen. Welche Faktoren diesen Zelltrieb regulieren, wissen wir nicht, vor allem nicht, warum er allmählich aufhört. Ob hier die Zeitdauer seiner Wirksamkeit, ob die erreichte Grösse des Individuums den Ausschlag für das Abklingen des Wachstumstriebes gibt, ist bis jetzt nicht entschieden.‡

Rubner has attempted to formulate its character:

> Die eine grosse Unbekannte auf dem Gebiete der Wachstumsphysiologie ist der *Wachstumstrieb*, der in gesetzmässiger Weise den Gang der Entwicklung, Massenzunahme, durch die Regelung der Ernährung leitet. Den *Urgrund* hat dieser Wachstumstrieb in der Geschwindigkeit der Kernteilung; wie wir noch sehen werden leitet sich hieraus der ganze Prozess des Stoffumsatzes ab. Die Kernteilungsgeschwindigkeit ist offenbar etwas der Spezies Eigentümliches, somit sind wir nicht in der Lage, vorläufig tiefer in dieses Problem vorzudringen.§

The second factor in growth, namely, *nutrition*, can be approached more easily by the experimental method. It is along this line that we have hoped, therefore, to be able to attack some of the problems of the relative value of the individual foodstuffs. It is well known that growth can be retarded by means involving the nutrition of the individual. Waters has well summarized the situation in these words:

> The upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period. Some of our approved theories have been so extreme as to hold, in effect, that the animal must grow at its maximum rate practically every

*Rubner: Archiv für Hygiene, 1908, LXVI, p. 82.
†Certain aspects are considered in C. S. Minot: The problem of age, growth, and death. New York, 1908.
‡Aron: Biochemische Zeitschrift, 1910, XXXp. 207.
§Rubner: Archiv für Hygiene, 1908, LXVI, p., 86.

day from birth to complete maturity in order to reach its normal size, or the full stature fixed by heredity. In other words, it is assumed that the animal has but one way of reaching its full stature and full development, viz., by developing to its upper limit through its entire growth period. This assumes that the organism is utterly incapable of compensating for any retarded development at any time in its growth period, either by a subsequently increased rate of growth, or by extending, even in the slightest degree, the growth cycle, much less by growing for a time at least when so sparsely fed that no gain in weight occurs.*

Rubner has expressed the rôle of nutrition in growth as follows:

Kann die Ernährung auch keinen Wachstumstrieb schaffen, so kann sie, wenn ungünstig und unzweckmässig, doch zu einem *Hemmnis* des natürlichen Wachstums werden. Wachstumsbehinderung ist innerhalb gewisser Grenzen noch keine Ursache einer Existenzgefährdung, ein Kind, dem die Nahrung normales Wachstum hindert, stirbt deswegen durchaus nicht, es holt später leicht wieder ein, was es versäumt hat . . . Nur das steht sicher, dass die Behinderung des Wachstumstriebes, wie dies wirklich vorkommt, nicht während der ganzen Wachstumsperiode andauern darf, da sonst allerdings die Grösse des Individuums dauernd Schaden leidet. Verlorene Körpergrösse in der Jugendzeit kann nach Vollendung der Wachstumsperiode nimmermehr abgeglichen werden . . . Eine *optimale* Ernährung, wie die *Wachstumsernährung* sein muss, stellt an die richtige Auswahl der Stoffe ganz andere Anforderungen als eine einfache Erhaltungsdiät.†

Obviously the energy problem plays an important part in the nutrition of growing animals. For the present we are primarily concerned with the qualitative aspects of the diet rather than the quantitative features of the food-intake. These two factors may at times stand in intimate relation to each other; improperly constituted food may, for example, modify the amount eaten and therefore the energy available for growth. As was intimated in our first report we have been able to arrest development in rats by feeding mixtures containing a single protein; but inasmuch as the food intake was not measured at that time, it was impossible to say whether the chemical character of the diet or a quantitatively inadequate food consumption was responsible for the dwarfing. The *fact* brought out was that in these young animals there could be a *maintenance of weight*, precisely as in older rats.

Waters has appropriately emphasized the necessity of a more exact definition of what is meant by *maintenance*, in contrast with growth. He writes:

It has long been assumed that the body of an animal, when supplied with only sufficient nutriment to maintain its weight, remains constant in composition and that no growth or production or change of any sort occurs.

*H. J. Waters: The capacity of animals to grow under adverse conditions. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 3.

†Rubner: Archiv für Hygiene, 1908, LXVI, pp. 82–83.

It is true that the term *maintenance* has been used somewhat loosely, but in general we have been in the habit of regarding the animal in maintenance when its live weight was constant. A more correct definition of the term would perhaps be to say that the animal was in maintenance when its body was in energy balance, but the live weight has been the conventional measure of our maintenance values.*

It is generally admitted that the proteins satisfy several functions in a growing organism as well as in the adult. The first is that of maintenance, corresponding with what has been termed the "Abnutzungsquote," or wear-and-tear, by Rubner. This makes good the inevitable losses occasioned by the processes of metabolism, cellular and secretory processes, etc. It is a small yet ever present need for protein (as well as energy), representing in a general way the minimal protein need of the stationary organism. Any excess of protein beyond this maintenance requirement may, in the adult, experience temporary storage ("Ansatz") or be devoted to dynamogenic purposes; but in the organism capable of development it contributes a share toward growth. It should be emphasized that the rate of growth is not by any means proportional to the excess of protein available. It is surprising, indeed, how small a content of protein in the dietary suffices to make growth possible. Rubner and Heubner† found, for example, that in suckling infants a protein intake equivalent to 5 per cent of the total calories satisfies the protein needs of maintenance, while 7 per cent permits of growth. Rubner writes:

> Das Wachstum ist eine *Funktion der Zelle,* es kann durch unzureichender Eiweisszufuhr *latent* werden, aber Eiweiss vermag *nicht* die Wachstumsschnelligkeit über die von der Natur gestreckten Grenzen zu heben, daher wird mit steigender Eiweissmenge in der Kost prozentisch weniger verwertet und das überflüssig zugeführte Eiweiss wird einfach als Brennstoff verbraucht der isodyname Mengen N-freier Stoffe einspart. Diese starke Anziehung von Eiweiss zum Wachstum nimmt im Laufe der Entwicklung ab und ist am grössten in der ersten Zeit des Lebens.‡

Waters has found in his extensive studies on cattle that growth, in the sense of changes of size and form, may occur even under adverse nutritive conditions. Fundamentally such investigations touch upon the much controverted question as to the relative importance of breeding and feeding in determining the shape and activities of mature animals. It is well known that by limiting the food supply of an ungrown individual, its development may be retarded. If the underfeeding is prolonged through the cycle of growth, the full stature limited by heredity may not be reached.

*H. J. Waters: The capacity of animals to grow under adverse condition. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 3.

†Rubner and Heubner: Zeitschrift für experimentelle Pathologie, 1905, I, p. 1.

‡Rubner: Archiv für Hygiene, 1908, LXVI, p. 110.

Waters asked the question:

Will this animal of smaller stature be in the same proportion with respect to all the organs and the different parts of its body as though it had been nourished to its full capacity and had attained its normal size and maximum development? Or will in this period of sparse nourishment a more complete development occur in certain parts of the body than in other parts? In short, when there is not sufficient food supplied to the growing animal to develop all of the organs and all parts of the body to their full limit and extent, will the rate of development of certain of these organs or parts diminish earlier than others and will the development of certain parts cease altogether before the development of other parts is diminished in rate and is it possible that some parts may cease their development before that of other parts?*

In actual experiments at the Missouri Agricultural Experiment Station, Waters found that ungrown cattle may remain at a constant body-weight for a long period of time, and yet increase in height and apparently decrease their store of fat. In other words, the skeleton has grown, or at least the bones have lengthened. Two interesting illustrative protocols† are reprinted here, one, Table XXXI, in which a stationary body-weight was maintained, the other, Table XXXII, in which there was actual decline on a starvation ration.

TABLE XXXI (FROM WATERS, TABLE II).—SHOWING INCREASE IN HEIGHT AT WITHERS, LENGTH OF HEAD, DEPTH OF CHEST, WIDTH OF CHEST, AND LOSS OF FAT IN A YEARLING STEER WHEN KEPT AT A STATIONARY BODY-WEIGHT.

No. 595. Grade Hereford. Born May 15, 1907. Nine and a half months old when experiment began. Full fed four months previous to beginning of trial. Condition when put on maintenance, medium. Weight at beginning of trial, 609.2 lbs. Weight at close of trial, 595.6 lbs. Average of ten daily weights.

Date.	Height at withers.	Length of head.	Depth of chest.	Width of chest.	Condition.
1908.	*cm.*	*cm.*	*cm.*	*cm.*	
Feb. 8	109	38	56	35	Medium.
Mar. 13	112.5	40	58	36.5	Medium.
Apr. 11	115.5	41	57.5	35.5	Medium to thin.
June 2	116	42	59	33.5	Common.
July 1	117.5	44	58.5	34	Common.
Aug. 1	117.5	44	59	33	Common.
Sept. 2	117.5	44	59.5	33	Common to fair.
Sept. 29	119	45.50	59.5	33.5	Fair.
Oct. 30	119.25	45.75	59.5	31	Fair.
Nov. 30	119.5	45.75	59.5	31	Fair to thin.
1909.					
Jan 1	119.75	46.50	60.5	30.75	Thin.
Jan. 30	119.75	45.50	60.75	30.75	Thin.
Total height in 12 months.	10.75	7.50	4.75	*− 4.25	
Per cent gain	9.86	19.73	8.48	*−12.1	

NOTE.—When slaughtered, carcass was classed as poor canner. All visible subdermal and intramuscular fat had disappeared.

*—Denotes a loss.

*H. J. Waters: The influence of nutrition upon the animal form. Proceedings Society for the Promotion of Agricultural Science, 1909, xxx, p. 71.

†From H. J. Waters: The capacity of animals to grow under adverse condition. Proceedings Society for the Promotion of Agricultural Science, 1908, xxix.

TABLE XXXII (FROM WATERS, TABLE VI).—SUB-MAINTENANCE.

Steer No. 591. Grade Hereford. Born May 15, 1907. Experiment began Feb. 26, 1908.
Age of animal at beginning of experiment, nine and a half months.
Full fed four months before trial began and was in good condition.
Weight at beginning of trial, 572.7 lbs. Weight at close of trial, 490.4 lbs.
Total loss in weight, 82.3 lbs. Average daily loss 0.43 lb. —Denotes loss.

Date.	Height at withers.	Length of head.	Depth of chest.	Width of chest.
1908.	*cm.*	*cm.*	*cm.*	*cm.*
Feb. 8	110.5	39	57	38.5
Mar. 13	113	41.5	57.5	34.5
Mar. 28	115	42	....	35
Apr. 11	114.5	41	58	33
May 2	116	42	57	33
June 1	118.5	44	57.5	33
June 29	120	44	58	31.5
July 31	119	44.5	59.5	29.5
Aug. 31	119.5	44.5	58	29
Gain	9	5.5	1	− 9.5
Per cent	8.14	14.10	1.75	−24.6

The following is from Waters, in regard to a series of comparable cattle maintained by him on different nutritive planes, designated as sub-maintenance, maintenance, and super-maintenance:

It is to be observed that there is no appreciable difference in the rate of growth in height of these three animals on widely different nutritive planes, from the beginning of the experiment (February) to the end of June. At this time the curve of the sub-maintenance animal flattens perceptibly. A month later, the maintenance animal is apparently responding to the influence of the low nutritive plane. As would be expected, in the case of the super-maintenance animal, the rate of growth remains unchanged. It may be surprising to many [Waters writes elsewhere] that an animal on maintenance, much less on sub-maintenance, should show any increase whatever in the width of hip or length of leg . . . Apparently the animal organism is capable of drawing upon its reserve for the purposes of sustaining the growth process for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a considerable extent to support growth the process ceases, and there is no further increase in height or in length of bone. From this point on the animal's chief business is to be to sustain life. This law applies to animals on a stationary live weight as well as those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, become thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.*

More recently Aron† has made comparable studies on growing dogs. He formulated his problem in the following words:

"Was wird geschehen, wenn für kürzere oder längere Zeit in der Nahrung nur so viel Energie usw. zugeführt wird, wie erforderlich ist, um den Erhalt-

*H. J. Waters: How an animal grows. Kansas State Board of Agriculture, Seventeenth Biennial Report, 1909–1910, I, p. 208.

†Aron: Biochemische Zeitschrift, 1910, XXX, p. 207.

ungsbedarf des wachsenden Organismus zu befriedigen, aber kein Ueberschuss, der als Wachstumsenergie dienen könnte? Die nächstliegende Annahme ist, dass dann kein Wachstum stattfindet, dass der Wachstumsprozess stillsteht. Können wir nun wirklich den Wachstumstrieb durch Nahrungsbeschränkung unterdrücken? Wie lange? Und was geschieht später mit einem wachsenden Organismus, dessen Wachstum eine Zeitlang hintan gehalten worden ist? (p. 208.)

Aron succeeded by restricted feeding in attaining constancy of body-weight in practically all of his dogs, in some cases during a period of nearly a year. The daily gains or losses fluctuated within a few grams. The description of the animals during the experiments is of interest to us:

Bei allen Hunden konnte man deutlich beobachten, wie die Tiere trotz des Gewichtsstillstands *wuchsen*, d.h. an Höhe und Länge zunahmen. Dabei wurden die Tiere zusehends magerer, Fett und Muskeln schienen an Masse abzunehmen, die runden Formen schwanden, die Knochen traten eckig unter der Haut hervor, und schliesslich schienen die Tiere nur noch aus Haut und Knochen zu bestehen. Trotzdem waren die Hunde nicht etwa schwach. Im Gegenteil, sie waren lebhaft, liefen und sprangen umher, oft mehr als ihre normalen Brudertiere, die ein zwei- oder dreimal zo grosses Körpergewicht zu bewältigen hatten. Dieser Zustand zunehmender Abmagerung unter ständiger Grössen-, d.h. Längen- und Höhenzunahme bei Konstantbleiben des Gewichtes dauerte je nach dem Grade der Nahrungsentziehung ungefähr 3 bis 5 Monate an. Wurde jetzt, wenn das Tier völlig abgemagert war, . . ., die Nahrungsmenge *weiter so gering* belassen wie vorher, so ging das Tier unter geringem Gewichtsverlust in völliger Inanition zugrunde. Wurde aber jetzt die Nahrungsmenge etwas erhöht, wie bei Hund A, so hielt sich das Tier zwar vollkommen abgemagert, aber auf konstantem Gewicht. *Und jetzt erweist sich dieser Gewichtsstillstand als identisch mit Wachstumsstillstand!* Der Hund A ist noch weitere 5 Monate auf dem gleichen Gewicht gehalten worden, ohne dass sich nun in seinem Aussehen nennenswerte Aenderungen konstatieren liessen.

Durch geeignete Nahrungsbeschränkung gelingt es also, wachsende Hunde beliebig lange auf konstantem Gewicht zu halten. Natürlich darf man nicht allzu junge Tiere nehmen. Während dieses Gewichtsstillstandes gehen aber gewaltige Umwandlungen im Tierkörper vor, die sich äusserlich in dem fortschreitenden Längen- und Höhenwachstum und der Abmagerung dokumentieren.

Offenbar ist trotz des Gewichtsstillstandes das Skelett weiter gewachsen und hat nicht nur an Grösse, sondern auch an Masse zugenommen. Folglich müssen andere Körpergebilde (wie Haut, Fleisch, Organe usw.) an Gewicht verloren haben; denn sonst könnte ja das Gewicht des Tieres nicht das gleiche geblieben sein. Ebenso wie die Massenverhältnisse der einzelnen Körpergebilde haben sich nun höchstwahrscheinlich auch die Mengenverhältnisse der einzelnen Körperbestandteile, wie Fett, Eiweiss usw., beträchtlich verschoben. (p. 212.)

Aron's analyses of the underfed dogs showing stationary weight in comparison with well-fed control animals indicate that in addition to the bones, the brain also was protected from loss of weight, while the adipose and muscular tissue suffered notable losses. Most striking is the degree to which water has replaced the tissue substance

utilized to compensate for the lack in the food, the blood especially becoming distinctly "watery," as the selected protocol shows:*

TABLE XXXIII.—CONTENT OF DRY MATTER IN VARIOUS TISSUES.

	Control dog.	Underfed dog.
Blood	*18.8	*5.1
Brain	24.6	19.3
Bones	57.2	40.0
Muscle	29.1	15.2

*Protein = N×6¼.

It is apparent here, as in Waters's experiments, that the energy deficit has been furnished by the body. "Sind alle verfügbaren Reservestoffe aufgebraucht, dann gewinnt der Erhaltungstrieb die Oberhand über den Wachstumstrieb, und das 'Wachstum' stockt." (Aron, p. 222.)

In relation to our own later observations it is desirable to quote Aron's view regarding the impulse to growth. He concludes:

. . . dass die innere treibende Kraft zum Wachsen überhaupt in dem Kerngerüst des Körpers, dem Skelett, ruht. Die Muskulatur verfügt anscheinend über gar keinen richtigen Wachstumstrieb. Sie folgt dem wachsenden Skelett nur dann, wenn die Ernährungsverhältnisse es erlauben, vielleicht auf Grund rein mechanischer Kräfte (Zug).

Recht interessant scheint zum Schluss noch die Frage, wie sich bei den durch lange fortgesetzte Unterernährung im Wachstum zurückgehaltenen Tieren die Entwicklung und die Entwicklungsfähigkeit verhält. Mein Tiermaterial war nicht ausreichend, um ein Studium der Geschlechtsorgane der zwar im Alter der Geschlechtsreife stehenden, aber im Wachstum weit zurückgebliebenen Tiere zu gestatten. Dagegen scheint mir die Beobachtung der *Stimme* auf ein wirkliches Zurückbleiben der Entwicklung auf dem infantilen Stadium zu deuten. Die Unterschiede zwischen den Brudertieren der ersten, zweiten und vierten Versuchsreihe waren auffällig. Die im Gewicht zurückgebliebenen Tiere schrien kreischend wie junge Hunde, während ihre normalen Brudertiere mit tiefem Tonfall bellten. In ganz dem gleichen Sinne spricht die von Waters festgestellte Tatsache, dass seine in Gewicht und Wachstum zurückgebliebenen Tiere ein Fleisch, das für 'Kalbfleisch' charakteristisch war, aufwiesen, während sie dem Alter nach schon "Rindfleisch" besitzen sollten. (pp. 222–223.)

Studies of the relation of weight to the measurements of children during the first year† have also given evidence of "disproportionate" growth in the case of poorly nourished infants. Whereas there is, in the normal infant, a fairly constant relationship between body-weight and height, circumference of head, chest, etc., this is not true where proper increase of body-weight is retarded by poor nutrition. For example, in children whose weight at the end of the third month

*Aron: Biochemische Zeitschrift, 1910, xxx, p. 220.
†E. C. Fleischner: Archives of Pediatrics, October 1906.

is only equal to that of a normal child at birth, the height has been found above that of the latter, illustrating, as Fleischner remarks, "that age plays some part in the growth of the infant, independent of the weight." This corresponds with the cases of the animals already cited. Fleischner concludes from his measurements of 500 children of whom 25 per cent were well nourished, 35 per cent fairly well nourished, and 40 per cent poorly nourished:

> It is in the poorly nourished children that age plays its most important part . . . In the poorly nourished children, most of whom are probably somewhat premature, when the weight is below normal, all the measurements are correspondingly below normal. The height and circumference of the head reach the normal birth measurements a little ahead of the weight, while the chest and abdomen are two months later in reaching the measurements of a normal child at birth. When the weight is stationary the increase in the measurements is very small, depending upon the slight influence which age has upon the growth of the infant notwithstanding the weight. The measurements of infants of the same weight, notwithstanding the age, are very similar, the small difference depending, as when the weight of a child is stationary, upon the very slight influence of age upon growth. The final conclusion can be drawn that during the first year of life the primary factor in the increase of the measurements of the body is steady, consistent increase in the weight, the influence of age being secondary and much less important.*

SUSPENSION OF GROWTH ON A MAINTENANCE DIET.

Early in the course of our investigation we noted that young rats could remain in apparent good health while living on some of the mixtures of isolated food-stuffs, without giving any evidence of growth. In some instances the animals ultimately declined and died where the diet was not changed; but in numerous cases body-weight, which we used as our guide, remained practically unchanged or showed a minimal slow increase (cf. Charts XXXVII, LXIII, and LXIV). The experiment showing the greatest growth under these dietary conditions is recorded in Chart XXXVIII. Other investigators have met with this stationary condition and accepted it as evidence of satisfactory nutritive equilibrium. We soon became convinced, however, that a diet which will not induce real growth at the proper age is unquestionably defective from the standpoint of perfect nutrition. Furthermore, inasmuch as the ungrown rat has a far smaller reserve of available energy and manifests the utilization of a suitable diet both speedily and conspicuously by its measurable changes in size, the animal becomes an exceptionally appropriate subject at this early stage for the study of the nutritive requirement.

The most precise evidence which we can present at this time of the stationary condition of the animals which we have stunted by

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*E. C. Fleischner: Archives of Pediatrics, October 1906.

the particular dietaries adopted is derived from measurements on three young rats of the same litter maintained for 124 days without noteworthy growth, on a diet of

	Per cent.
Glutenin	18.0
Starch	14.5 to 34.5
Sugar	15.0 to 20.0
Agar	5.0
Salt mixture I	2.5
Lard	20.0 to 45.0

The curves of growth of these animals as well as three others from the same brood fed on mixed food or the milk-food mixture (and showing a normal growth) are reproduced in Charts LXXXI, LXXXII, LXXXIII, LXXXIV, LXXXV, and LXXXVI.

The animals were killed at the age of 178 days and measurements were made by Dr. S. Hatai, of the Wistar Institute. The tabulated data are given on the following page, together with a report from Dr. Hatai, to whom, as well as to Dr. Donaldson, we are greatly indebted for helpful cooperation.

The statistics of body-length, weight of brain, spinal cord, etc., of the stunted animals at an age of 178 days are comparable with those characteristic for normally growing rats of the *same* body-weight, which is attained at an age of approximately 54 to 63 days. Here, then, are illustrations of maintenance without growth.

Dr. Hatai further reports as follows:

Since it seems to be the least variable character, I have selected the body-length as the basis for computation. When the other characters which we can measure are calculated from the formulas based on body-length, it is seen that the observed weight of the brain and of the spinal cord agrees closely with the calculated in both the control and the stunted rats. Thus both series have a growth of the nervous system normal to their body-length. In the control series, the percentage of water observed in both the brain and the spinal cord agrees with that calculated according to the body-length. In general then the control rats agree with the general population in these characters. Since the stunted rats have an abnormally small body-length for their age, they can not be treated by the formula for determining the percentage of water from body-length. When, however, we take the estimated percentage of water for 178 days (see Donaldson*) we find that this value agrees with that observed in the stunted series. It may be further noted that the ratio between body-length and tail-length is the same in both series. We therefore conclude that *in both series the body-weight is normal to the body-length; the brain and spinal cord weight normal to the body-length; and the percentage of water normal for age.* Concerning other organs we have no data, but we may infer from the foregoing that they also have weights normal to the body-length. You will see from the above that the stunted rats though small have the general relative development of the controls and that in the only case where it is possible to follow the maturing process, that is in the percentage of water in the nervous system, they have matured in accordance with their age (see Donaldson*).

*Donaldson: Journal of Comparative Neurology, April 1911.

A

B

C

A. Rat 238, female. Age 140 days, weight 144 grams, which is normal for a rat of same age as 240.
B. Rat 240, female. Age 140 days, weight 55 grams. Same brood as Rat 238.
C. Rat 305. Age 36 days, weight 55 grams. Showing the appearance of a normal rat of same size as 240.

A and B show the contrast between two rats of the same age, one of which (Rat 240) has been stunted. The lower two pictures afford a comparison between two rats of the same weight, but widely differing in age. The older, stunted rat, B, has not lost the characteristic proportions of the younger animal, C.

D

E

F

D. Rat 168, male. Weight 235 grams, which is normal for a rat of the age of 220 shown below.
E. Rat 220, male. Age 148 days, weight 58 grams.
F. Rat 305. Age 36 days, weight 55 grams. Showing appearance of a normal rat of same weight as 220.
D and E show the contrast between two rats of the same age, one of which (Rat 220) has been stunted. The stunted rat is not essentially altered in its bodily proportions from those of a much younger rat of the same weight.

TABLE XXXIV.—HATAI'S MEASUREMENTS OF STUNTED RATS FROM EXPERIMENTS OF OSBORNE AND MENDEL, 1910–1911.

CONTROL RATS.

	Diet.	Sex.	Age in days.	Weight in grams of—			Hypophysis.	Percentage of water.		Length in mm. of—	
				Body.	Brain.	Cord.		Brain.	Cord.	Body.	Tail.
Rat 96...	Milk	Fem.	178	154.9	1.7346	0.4934	0.0073	78.306	71.139	176	146
Rat 97...	Milk	Fem.	178	164.5	1.6974	0.5007	0.0093	78.473	71.220	183	164
Rat 99...	Mixed	Male	178	175.0	1.8515	0.4810	0.0052	78.623	71.809	181	144
Average..	....	....	.....	164.8	1.7612	0.4937	0.0071	78.467	71.389	180	151
Calculated from body-length.........					1.7645	0.5004	......	78.374	71.192	180	.....
Estimated percentage of water from age					......	......	......	78.4	71.2	.....	.....
Body-length to tail-length 1 : 0.83											

STUNTED RATS.

	Diet.	Sex.	Age in days.	Body.	Brain.	Cord.	Hypophysis.	Brain.	Cord.	Body.	Tail.
Rat 100..	Glutenin	Fem.	178	85.0	1.6323	0.4089	0.0035	78.141	70.775	148	129
Rat 101..	Glutenin	Male	178	71.8	1.5022	0.3781	0.0022	78.272	71.701	139	108
Rat 102..	Glutenin	Male	178	85.7	1.6280	0.3977	0.0033	78.133	71.134	148	125
Average..	....	....	.....	80.8	1.5875	0.3929	0.0030	78.182	71.203	145	121
Calculated from body-length..........					1.5896	0.3639	......	......	......	145	.....
Estimated percentage of water from age					......	......	......	78.4	71.2	.....	.....
Body-length to tail-length 1 : 0.83											

FORMULAS.

Brain weight $=0.569 \log \left(10\frac{\text{Body-length}+134}{143}-23.7\right)+0.554$

Spinal cord weight $=0.585 \log \left(10\frac{\text{Body-length}+134}{143}+6\right)-0.795$

Percentage of water (brain) $=82.62-2 \log (\text{Body-weight}-10)$.
Percentage of water (spinal cord) $=85.20-6.5 \log (\text{Body-weight})$.

Photographs of other rats which have been dwarfed in like ways give evidence of the similarity of the stunts in general appearance with normal animals of the same weight at a much earlier age. Thus, in Plate 1, rat 305, C, weighing 55 grams at the age of 36 days, compares favorably with rat 240, B, dwarfed on a gliadin food mixture, at the age of 140 days, when it weighed 55 grams (cf. Chart CXIII). It is interesting to contrast B with the uppermost photograph A of rat 238, likewise 140 days old and from the same brood but weighing 146 grams, the normal weight for this age. Each was raised under

identical conditions from the age of 38 days, except that rat 238 (see Chart LVI) was fed with a paste containing *casein* and protein-free milk, while in the food of 240 (see Chart CXIII) the casein was replaced by *gliadin.*

Plate 2 shows rat 220, E, fed on gliadin and protein-free milk but weighing only 58 grams, although 148 days old, and, for contrast, rat 168, D, of approximately the size normal for the age of rat 220, is also shown. Figure F shows a normally nourished rat of the same weight as rat 220. This picture is introduced to show that rat 220 has the appearance of a normal rat of corresponding size and weight. All these pictures were taken on exactly the same scale and afford a ready comparison of the relative sizes of the animals.

The interesting photographs of underfed cattle published by Waters, on the contrary, make the change of form in his undernourished animals of stationary weight quite apparent. We are, however, not prepared to assert that careful measurements of our stunted rats will not disclose some trace of similar changes in skeletal form. They must be slight at most; for we have often compared animals long maintained at small stature with properly grown animals which have just reached the same weight, without detecting any deviation from the youthful form in so far as one could judge by mere visual inspection. The photographs speak in the same sense.

The point on which we lay great stress in the foregoing experiments is the fact that the stunting is not attributable primarily to *under-feeding.* Our dwarfed rats have as a rule eaten as adequately as normally nourished animals *of the same size.* The energy factor, as such, thus drops out of the problem. In this respect the experiments are not comparable with those of Waters and of Aron, both of whom accomplished their results by underfeeding with adequate food materials. In our experiments the "energy requirement for maintenance" and the "energy requirement for growth," which together are essential to the developing organism, were both supplied. The rats did not grow primarily at the expense of stored tissue materials: they failed to grow in any sense. *We are obviously dealing with some other feature than insufficient energy supply.* The numerous illustrative experiments which will be cited later are accordingly to be interpreted as instances of *maintenance* without growth. If it is true that growth can only continue when the energy intake exceeds the mere maintenance requirement, it is equally true that an excess of calories does not *per se* insure growth in a suitable animal. Here then is the opportunity to ascertain and differentiate some of the essential qualitative factors: protein, inorganic salts, etc.—their minimum and optimum values.

EFFECT OF STUNTING ON THE GROWTH IMPULSE.

Before proceeding to study the influence of dietary variations on (*a*) maintenance and (*b*) growth, respectively, it became necessary to learn whether a more or less temporary inhibition of growth checks or in any degree modifies the capacity to grow (Wachstumstrieb). The literature on this subject by no means reveals a unanimity of opinion, although familiar experience will bring to mind many illustrations of compensated retardation of growth in children.* A few typical experiments may be cited. Rat 36 (male) kept stunted 49 days on a diet of gliadin food† (37 days) followed by casein food mixture† (12 days), showed complete recovery of growth on a mixed diet (see Chart XCVI). The "*mixed diet* of our animals consists of dog biscuit, sunflower seed, and fresh carrots (with occasional changes and addition of lumps of rock salt). Rat 65 (female) stunted, during 33 days on a diet of casein-zein food,‡ likewise resumed a normal rate of growth as soon as the mixed diet was instituted (see Chart XXXVII).

Special interest is attached to experiments in which after a preliminary stunting period the resumption of growth was accomplished on a diet containing milk as the effective component. Two protocols of the diet during the stunting period are reproduced in Table XXXV, with reference likewise to Charts XXVIII and XXIX.

TABLE XXXV.

Duration of stunting.	Rat 64 (female), 33 days.		Rat 51 (male), 46 days.	
		per cent		*per cent*
Stunting diet..........	Casein	12.0	Casein	18.0
	*Zein	6.0	Starch	29.5
	Starch	29.5	Sugar	15.0
	Sugar	15.0	Agar	5.0
	Agar	5.0	†Salt mixture I	2.5
	†Salt mixture I	2.5	Lard	30.0
	Lard	30.0		

*The zein was hydrated by the addition of a little water. †Cf., p. 86.

The curves in these cases are seen to be quite comparable with those of the normally growing rats. Bearing in mind that the animals here studied were continually kept in small cages under actual experimental conditions, the "normal" character of the growth curves makes it evident that the environment is no wise detrimental.

*Cf. Condereau: Recherches chimiques et physiologiques sur l'alimentation des enfants, Paris, 1869; Pagliani: Giornale della reale societa italiana d'igiene, Milano, 1879, I. (Quoted by Hatai: American Journal of Physiology, 1907, XVIII, p. 320.)
†See p. 122.
‡See p. 98. Water was added to this mixture until the zein was well hydrated.

Normal growth, as judged by curve of increase in body-weight, was resumed on a diet consisting of

	per cent.
"Trumilk"	60.0
Starch	16.7
Lard	23.3

Similar experiences are shown after feeding gliadin (Charts XCIX, C) or edestin (Chart LXV).

In the case of rat 37 (Chart XCVII), a stunting period of 49 days on a diet of gliadin food for 37 days, followed by casein food mixture for 12 days, was followed by normal resumption of growth under a dietary régime in which a period of feeding on the above milk-food was alternated with mixed food. Judging by the typical character of the curve of growth in this animal the two types of resuscitation diet, though radically different in origin, are equally efficacious in promoting growth. The growth curve shows little deviation from its usual course incidental to the changes in the dietary.

It may be remarked that the early stunting does not necessarily impair the capacity to breed at a later period when growth is again established. Furthermore, we have found that the milk-fat-starch mixture continued from early life in no wise impairs the potency of rats as breeders. Its nutritive efficiency will be referred to again.

Experiments such as those recorded above give unmistakable evidence of the fact that a considerable period of stunting by no means impairs the "Wachstumstrieb" of these animals. As soon as an appropriate diet is instituted growth begins anew and proceeds with practically the same speed as under normal conditions. By this we mean that a definite increment of gain from some fixed weight requires approximately the same period for its accomplishment as in the case of uninterrupted growth. A rat which will ordinarily grow from 60 grams to 180 grams in body-weight in 60 days will make the same gain even when its growth has been inhibited days or even weeks and its size and form retained at a maintenance level. This will be apparent by comparing, for example, the normal growth curve for both male and female rats with that of the realimented rats, during the same period of time, in Charts CXXII and CXXIII.

It should be emphasized that the situation is here quite different from that developed by Waters and Aron in the experiments on cattle and dogs. With their conditions of underfeeding the animals increase in size (height, etc.) while starving; and during the earlier period of such trials a poorly fed animal may actually gain in height as rapidly as a highly nourished one, fed to the limit of its appetite.*

*Cf. Waters: The capacity of animals to grow under adverse conditions. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 15.

The duration of the period of growth of the undernourished animal depends upon the constitutional vigor of the individual and the store of fat which it has accumulated. Quoting Aron: "Dem Einschmelzungsprozess fällt neben dem Fettgewebe in erster Linie die Muskulatur zum Opfer, während die Organe ihm widerstehen, wohl weil sie lebenswichtiger sind."

The results of realimentation in animals which show this "disproportionate" growth, *i. e.*, growth of one part at the expense of another, are not yet satisfactorily ascertained. Waters believes that physiological compensation may result "by an increase in the rate of growth in a period of liberal feeding following a period of low nourishment and low gain. In other words, an animal that is below the normal in size at a given age, through poor nourishment, apparently has the capacity, when liberally fed, to compensate for this loss, in a measure at least, by an increased rate of gain." He also suggests the possibility that growth may be accomplished on a more economical basis—a view which we are not yet ready to accept.

EFFECT OF PARTIAL STARVATION ON BODY-WEIGHT.

Hatai* has studied the effect of partial starvation followed by normal diet on the growth of white rats. The "partial starvation" consisted in feeding a diet that is practically devoid of protein, viz, starch and water, during 21 days to animals about 40 days old. The realimentation was continued to the age of maturity, at the end of 200 days. The statistics thus obtained and reproduced in Table XXXVI are presented graphically in Chart XXVI.

TABLE XXXVI.—HATAI'S MEASUREMENTS OF UNDERFED AND REALIMENTED RATS.

	Body-weight.			Total gain.	Ratio between initial and final.
	Initial.	After 21 days.	Final.		
	gm.	*gm.*	*gm.*	*gm.*	
Male, controls	35.2	63.1	224.4	189.2	1 : 6.37
Male, experimented	37.6	28.4	242.0	204.4	: 6.43
Female, controls	36.3	67.8	†172.6	136.3	: 4.75
Female, experimented	34.3	27.0	†167.8	133.5	: 4.89

Hatai concluded that, as far as body-weight is concerned, "the experimented rats have completely recovered from the effect of 21 days of partial starvation . . . The recovery in the weight is most astonishing, especially during the first 3 or 4 days, within which time the starved rats regain the weight lost during the 21 days of starvation. Later the increase in weight is very steady, though not as rapid as during the first few days, until the rat has reached the age

*Hatai: American Journal of Physiology, 1907, XVIII, p. 310.
†The body-weight in both control and experimented is small for the age.

of 150 days, and after this age increase in weight is relatively slow. What will happen to such rats during the later portions of the span of life has yet to be determined in order to answer the question whether this partial starvation in early life has any influence either on longevity or the onset of old age." (p. 314–315.)

EFFECT OF PARTIAL STARVATION ON NERVOUS SYSTEM.

Though the period of retarded growth was eventually completely compensated in Hatai's animals, in so far as the weight of the body and central nervous system are concerned, the chemical composition of the brain and spinal cord was not entirely free from the effect. As the result of an extended investigation of the effects of underfeeding on the nervous system, Donaldson* has arrived at the conclusion that one of the characteristics of growth, the change in the water content of the brain, has not been arrested like the increase of the animal in size and body-weight, but apparently accelerated. He states:

> The underfed group are in this character similar to somewhat older animals. Evidence further points to the continued formation of the medullary sheaths with advancing age even in rats which are underfed, *i. e.*, underfeeding does not arrest medullation. Underfeeding which stops growth of the body and retards that of the nervous system does not modify the percentage of water in the spinal cord, while it does reduce it in the brain—the amount of this reduction being less in the cases where the underfeeding is less severe.†

With respect to the possible psychological effects of such underfeeding and return to normal diet Donaldson says:

> So far as our tests show, such an experience does not modify the rat's ability to learn, for, by a series of experiments, it has been possible to determine that such a rat can learn to get its food under complicated conditions just as well and as rapidly as a normal animal (Hayes).‡

The preceding facts as to resuscitated rats are recorded here—despite the fact that this temporary stunting was produced by underfeeding (rather than unsuitable feeding as in our experiments)—because they suggest that the real story of the condition of the animals may perhaps not be revealed by the external evidences of growth. It is not at all impossible that the rats which we have dwarfed for months may have experienced some continued subtle changes in the make-up of the nervous system despite the appearance of unchanged youth which they manifest. Measurements of size and weight alone may not suffice to disclose the real physiological status of the animal, especially in respect to the development of the nervous functions and structures, which are singularly pro-

*Donaldson: Journal of Comparative Neurology, 1911, XXI, p. 139.
†Donaldson: *ibid.*, p. 169.
‡Donaldson: Journal of Nervous and Mental Disease, 1911, XXXVIII, p. 262.

tected even during starvation. This is seen to be true in the series of stunted animals fed on the glutenin mixture in our experiments (p. 72). There is a large field of investigation still open here with important bearings on the problems of retarded growth in man. According to Donaldson* "the progressive diminution of the percentage of water in the central nervous system with advancing age is to be regarded as an index of fundamental chemical processes, which take place in the more stable constituents of the nerve cells. These processes are but little modified by changes in the environment and taken all together constitute a series of reactions which express not only the intensity of the growth process in the nervous system, but also the span of life characteristic for any given species." Possibly, then, the further study of the nervous system in connection with our experiments may throw light on the phenomena of malnutrition (which our stunting experiments primarily represent) as well as those of undernutrition or starvation.

It may be well here to note that the experience of Donaldson† indicates the main features of human growth to be well represented in the albino rat. So good is the essential correspondence that there is every reason to continue the work on this form. The striking difference is that the rat grows some thirty times as rapidly as man.

COMPARISON OF MILK AND MIXED DIET.

The failure either to induce substantial growth in young rats or to satisfy completely the maintenance requirement of older animals during very long protracted periods on the mixtures of isolated foodstuffs thus far reported raises the question as to what constitutes an ideal nutriment for a rat. The suitability of mixed diet is beyond question. The favorable experiences with dried milk powder (some of which have been recorded on pages 75 and 76) early directed our attention to this product. Rats were not only resuscitated after nutritive decline and suitably maintained, but also grown from early age on pastes in which the milk powder (with lard and starch) constituted the mixture. The commercial brand "Trumilk"‡ employed by us has been analyzed at the Connecticut Agricultural Experiment Station with the following results:

	Per cent.
Water	3.8
Total solids	96.2
Protein (N×6.38)	25.6
Fat	27.4
Lactose	37.2
Ash	6.0

*Donaldson: Journal of Comparative Neurology, 1910, xx, p. 143.

†Cf. Donaldson: Journal of Nervous and Mental Disease, 1911, xxxvii, p. 258.

‡This product was kindly furnished to us in powder form by the Merrell-Soule Co., Syracuse, N. Y.

The preparation apparently contains a small excess of iron over that found in cow's milk—probably as a contamination from the desiccating process used. It is obtainable in easily manipulated form and with the addition of a small amount of nitrogen-free lard and starch forms a food paste readily consumed by rats. These pastes have been used, either with or without our earlier standard salt mixture (I),* as follows:

	Per cent.	*Per cent.*
"Trumilk"	60.0	60.0
Starch	16.7	15.7
Lard	23.3	23.3
Salt mixture I	0.0	1.0
	100.0	100.0
Nitrogen content	2.5	2.5

We have carried rats through the period of growth as well as pregnancy on this diet alone, from the time that they were removed from the mother (cf. Charts XXXI, XXXII, and XXXIII).

As a further illustration of the excellent nutritive properties and physiologically appropriate "combination" of food ingredients in the milk food-mixture, illustrative charts are appended to show the recovery of rats moribund after prolonged periods of malnutrition, with lack of inorganic salts in the dietary (Charts XXXIV and XXXV). Many similar illustrations might be reproduced, giving evidence of the perfect realimentation of rats by the use of the milk food (cf. Charts XXVIII, LXV, XCIX, and C).

Remembering that our earlier trials with casein, the chief protein ingredient of the milk powder, and with combinations of casein and other proteins were at best successful only in maintaining nutritive equilibrium—and that not indefinitely—and were never adequate for the manifestation of real growth, we directed our attention to the non-protein constituents of milk. After numerous failures to modify the inorganic and non-protein ingredients of our dietaries by altering the relation of proportions of the various ions as well as the character of the carbohydrates and fats, it occurred to us that the protein-free portion of the milk might give the clue to the successful feeding of proteins which did not appear to be the inefficient factors in our cases of malnutrition. Accordingly a product was prepared as follows:

Perfectly fresh centrifugated milk, nearly free from fat, was precipitated in lots of about 36 liters by diluting with 7 liters of distilled

*This mixture, prepared in imitation of Röhmann's successful product and empirically found by use to be the most satisfactory of the different combinations tried, has the following composition:

	Grams.		Grams.
$Ca_3(PO_4)_2$	10.0	Mg citrate	8.0
K_2HPO_4	37.0	Ca lactate	8.0
NaCl	20.0	Fe citrate	2.0
Na citrate	15.0		
			100.0

(Cf. our previous report, Feeding experiments with isolated food-substances, Publication No. 156, Carnegie Institution of Washington, p. 32.)

water which contained 1.64 c.c. of concentrated hydrochloric acid. The flocculent precipitate of casein was strained out on cheesecloth and the very nearly clear solution was filtered through a pulp filter. The filtrate, which at the most was very slightly turbid from suspended fat, was tested carefully by the alternate addition of dilute alkali and acid to determine whether any more casein could be separated from it. The addition of alkali caused a slight precipitate which did not increase on adding more alkali or dissolve on the addition of even relatively large amounts of alkali. This was presumably chiefly calcium phosphate. The addition of acid in no case caused any further precipitation. The filtered milk serum was then heated to boiling for a few minutes and filtered. The filtrate, which was in all cases water clear, was then neutralized to litmus with a dilute solution of sodium hydroxide and evaporated to dryness on a steam bath at a temperature of about 70°. The product thus obtained formed a friable, pale yellow mass which was easily reduced to a fine powder by grinding in a mill. Several grams of this powder were tested for protein by dissolving in about 30 c.c. of water containing a little hydrochloric acid and warming gently. The solution was then saturated with ammonium sulphate. The precipitate, which appeared to consist chiefly of calcium sulphate, was separated by centrifugation, dissolved in a little water, and potassium hydrate solution and copper sulphate added. The solution showed no evidence of the biuret reaction until it was saturated with potassium hydroxide and shaken with alcohol. It then separated into two layers, the upper alcoholic layer showing a slight but positive biuret reaction. Millon's reaction tried on portions of 2 or 3 grams of the substance did not give a positive reaction. Nitrogen determinations in several lots of the protein-free milk powder thus made showed them to contain 0.66, 0.59, 0.60, 0.72, 0.71, 0.67, 0.75 per cent of nitrogen. Munk* states that if the proteins of milk are precipitated by alcohol, or separated according to Hoppe-Seyler, from one-thirtieth to one-fifteenth of the protein remains dissolved. All the proteins can be precipitated only by tannin in the cold or by copper hydroxide on heating. He further states that cow's milk contains about one-sixteenth of its nitrogen in non-protein form. Since our protein-free milk powder was equal to 50 per cent of the total solids of the milk, it should, if Munk's statements are correct, contain 0.48 per cent of non-protein nitrogen, thus leaving at the most only 0.28 per cent of protein nitrogen, equal to 1.69 per cent of protein. Since 100 grams of the food mixture employed in our experiments contained 28.2 grams of protein-free milk powder, we can assume that at the most the food pastes thus made contained only 0.48 per cent of milk protein. The protein-free

*Munk: Virchow's Archiv für pathologische Anatomie, 1893, 134, p. 501.

milk powder thus produced as above described left about 14.5 per cent of inorganic matter on ignition. This includes not only the inorganic constituents of the milk, although by no means in the combination in which they occur in the mammary secretion, but also the inorganic salts which were formed by the addition of the hydrochloric acid used to precipitate the casein and also the sodium salts which resulted from neutralizing the milk serum with sodium hydroxide solution.

EXPERIMENTS WITH ISOLATED PROTEINS AND "PROTEIN-FREE" MILK.

The use of this product (which we shall designate as protein-free milk) as an adjuvant to isolated proteins to furnish the inorganic elements of the diet has succeeded beyond our expectation. When employed, for example, in combination with various proteins, in the proportion in which its ingredients occur in the complete milk food already used (see page 76), it induces normal growth. Added during the periods of nutritive decline to food mixtures which no longer suffice to maintain rats, recovery has manifested itself in practically every case. Where, as in the case of zein, gliadin, or hordein feeding, no advantage has been obtained by the use of the protein-free milk, it has become obvious that the protein *per se* is the defective food constituent. Thus at length we have found a method of controlling or furnishing some of the most essential non-protein factors in the diet, so that the value of the individual proteins can be investigated under much more favorable conditions than formerly.

Numerous charts (see p. 103 fig.) present the graphic records of feeding experiments with casein, edestin,* glutenin,* glycinin,* gliadin,* hordein,* ovalbumin,† and lactalbumin,‡ showing appropriate growth, or maintenance, according to the age at which the animals were started on the use of the protein-free milk as the non-protein component in place of the earlier inorganic salt mixture.

It might be objected, after superficial consideration of these results, that the favorable outcome (especially for growth) is due to milk protein contaminating the "protein-free milk" component of the diet. Aside from the fact that the amount of possible contamination is at most small, evidence of the untenability of such a theory is available from several sources. In the first place, growth has not followed the use of *all* proteins when the protein-free milk was added to them.

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*For the preparation of these vegetable proteins see T. B. Osborne: Darstellung der Proteine der Pflanzenwelt, Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1909, II, p. 270.

†This was prepared by Hopkins's method and was free from conalbumin. Cf. Osborne, Jones, and Leavenworth: American Journal of Physiology, 1909, XXIV, p. 252.

‡The preparation of this is described on p. 81.

The results can be grouped in two series, viz:

Diet = Isolated protein, protein-free milk, starch, agar, fat.

Group I.—Young rats.	Group II.—Young rats.
Active growth with— Casein (Charts XLVI, XLVII, LII, LIII, LIV, LV, LVI, LVII, LVIII, LIX, and LX. Ovalbumin (Charts XC and XCI). Lactalbumin (Charts XCII and XCIII). Edestin (Charts LXXI, LXXII, LXXIII, LXXIV, LXXV, and LXXVI). Glutenin (Charts LXXXVII, LXXXVIII, and LXXXIX). Glycinin (Charts XCIV and XCV).	Little or no growth with— Gliadin (Charts CVIII, CIX, CX, CXI, CXII, CXIII and CXIV). Hordein (Charts CXXIV and CXXV).

The failures in group II lead to the conclusion that the proteins, gliadin and hordein, are inadequate for the functions of growth. We are presumably dealing with a chemical inadequacy rather than any toxicity and consequent lack of growth, judging by the fact that the gliadin and hordein rats are maintained in good, nutritive condition even in the absence of growth. Their body-weight is scarcely changed at all. Without the use of the protein-free milk or fæces-feeding gliadin rats have usually declined (Charts XCVIII, XCIX, and C).

A second reason why the success of these trials is not due to the presence of possible minute contaminations with milk protein is discoverable in Charts XLIII, XLIV, XLV, XLVIII, XLIX, L, LI, CVIII, CIX, CX, and CXI. Here the addition of not inconsiderable portions (5 to 30 per cent) of the actual milk food to the earlier inefficient protein mixtures is incapable of bringing about growth in any degree equal to that at once initiated when the protein-free milk is added in relative abundance.

Further evidence that a trace of milk proteins is not responsible for the growth of the rats fed with mixtures containing our protein-free milk powder is furnished by experiments in which successively larger quantities of the milk food are added to the gliadin food. Here we see that growth gradually increases with the larger additions of the milk food, although with even as much as 30 per cent in the food the rate of growth is much below normal. With additions of 5 or even 20 per cent of the milk food, the rate of growth is very slow, as shown by Charts CIV, CV, CVI, and CVII. That this result is to be attributed to the proteins introduced in the milk food and not to a combination of a small quantity of milk proteins together with a sufficient quantity of the inorganic or other constituents of the milk is shown by experiments now in progress in which the addition of the milk food to the gliadin and protein-free milk food is producing normal growth. In this mixture we have all of the constituents of

the protein-free milk present in the same proportions as in our milk food, but less than one-third of the protein constituents of the milk. It is therefore evident that only a small proportion of the protein constituents of the milk are required to produce normal growth, and it may be assumed that the presence of a small quantity of milk proteins in our protein-free milk powder would manifest itself by at least some slight growth.

DISCUSSION OF THE RESULTS AND THEIR BEARINGS.

We have stated that by our plan a biological comparison of different proteins in respect to their rôle in growth can at length be made. Our work in this direction must be regarded as barely begun. Nevertheless it is of interest to speculate as to the indications already gained and the outlook for future work. A comparison of the two groups of proteins—those adequate and those inadequate for growth purposes—at once reveals the fact that the latter category comprises proteins (gliadin, hordein, zein) commonly spoken of as chemically "incomplete." They lack one or more of the amino-acid complexes which are obtainable from the so-called "complete" proteins. None of them furnish glycocoll or lysine, and zein in addition is devoid of tryptophane. By feeding relatively small quantities of proteins like casein with gliadin growth begins at once. Here we can determine the minimum of suitable protein to satisfy this growth requirement—a study already begun (cf. Charts CXX, CXXI, CXXII, and CXXIII). The addition of amino-acids to "complete," as it were, the inadequate proteins can now be studied amid controllable factors; the biological rôle of hydrolyzed proteins and the significance of complete hydrolysis or digestion in nutrition can be examined anew.

The experiences which have demonstrated the striking differences in value of the individual proteins and the small proportion of casein which suffices to induce growth instead of standstill (cf. Charts CXX, CXI, CXXII, and CXXIII, for example) emphasize the importance of the *purity* of the protein fed. We have devoted much labor and incurred a very considerable expense to obtain proteins in a form as uncontaminated as present methods will permit. The products used were as pure as one would expect them to be if employed for purposes of refined protein analysis. Had less perfect products been employed it is quite conceivable and indeed likely that the admixtures would have sufficed to alter completely the outcome of many experiments. For example, gliadin is prepared free from glutenin only be very careful purification methods; and although the nutritive properties of these two companion proteins are extremely unlike, as clearly indicated by our trials, a failure to effect a complete separation of a little glutenin from gliadin would have been sufficient to prevent the deficiencies of the latter from exhibiting themselves. Or

again, failure to purify carefully a protein like casein will vitiate the study of a problem like the synthesis of amino-acids. Pure casein is glycocoll-free; and the continued feeding of such a product as the sole protein of the dietary enables one to make deductions respecting the synthesis of glycocoll. The use of crude commercial protein preparations can never satisfy the requirements of refined study in this domain, where small effects continued over long periods are of great importance. We believe, therefore, that such considerations justify the energy and expense which have been put into the work.

In relation to the much-discussed problem of the relative value of organic *vs.* inorganic phosphorus in nutrition, our data after feeding phosphorus-free edestin to growing rats (cf. Charts LXXV and LXXVI) show a success quite as great as that with phosphorus-containing casein (cf. Charts LVI, LVII, LVIII, LIX, and LX). The animals must here have synthesized their phosphorus compounds from inorganic phosphorus. Whether milk production and other functions calling for such synthetic reactions will continue adequately is open to investigation. It is also noteworthy that all of our animals grow on a dietary that is purine-free, or essentially so. Here the question of purine synthesis suggests itself. It is apparent, *e. g.*, in the case of gliadin, that the grown as well as ungrown rats may be *maintained* for long periods on single proteins.

With such an ideal non-protein dietary component at hand amino-acid substitutions can be attempted in the adult as well as in the growing animal. The protein minimum (or minima) is also open to accurate investigation. With a method of feeding devised which will permit a differentiation between growth and maintenance, which furnishes an energy-yielding protein-free component that is appropriate, and leaves the protein as the sole variable in the dietary, we believe that further contributions can be made to the problems of nutrition.

In the preparation of the large quantities of carefully purified proteins required for these experiments, we have been assisted by Mr. Charles S. Leavenworth, Mr. Owen Nolan, Mr. Leigh I. Holdredge, and Mr. Lawrence Nolan, whose valuable cooperation we are glad to acknowledge here.

THE CHARTS AND THEIR EXPLANATIONS.

In the following charts, to which reference is made in various places in the text, the abscissæ of the curves represent days and the ordinates actual body-weight (solid line) or food-intake (dotted line) in grams. In some of the charts the average (normal) curve of growth, plotted from body-weight data available for normally growing animals of the same sex, is represented by a broken line for comparison. The food-intake curve is plotted from the quantities of food eaten per week. The numbers on the body-weight curves indicate the time at which changes in the character of the feeding were instituted. All curves in this paper are plotted on the same scale, so that they are directly comparable.

Salt mixture I, to which reference is frequently made, was composed of—

	Grams.
$Ca_3(PO_4)_2$	10.0
K_2HPO_4	37.0
NaCl	20.0
Na citrate	15.0
Mg citrate	8.0
Ca lactate	8.0
Fe citrate	2.0
	100.0

INDEX OF CHARTS WITH REFERENCE TO FOOD-MIXTURES AND PROTEINS FED.

[Numbers refer to pages in the text.]

CHART XXII.

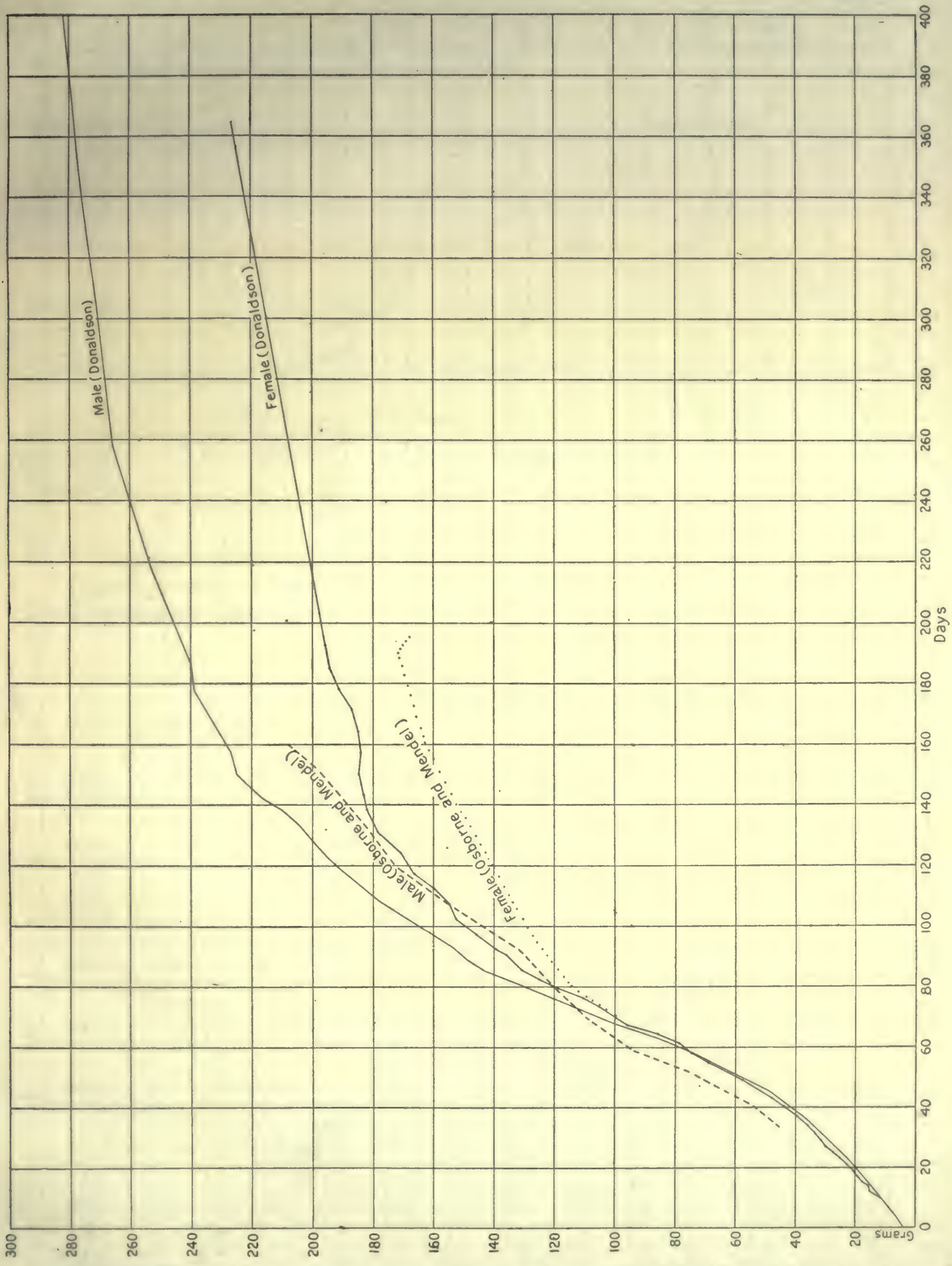
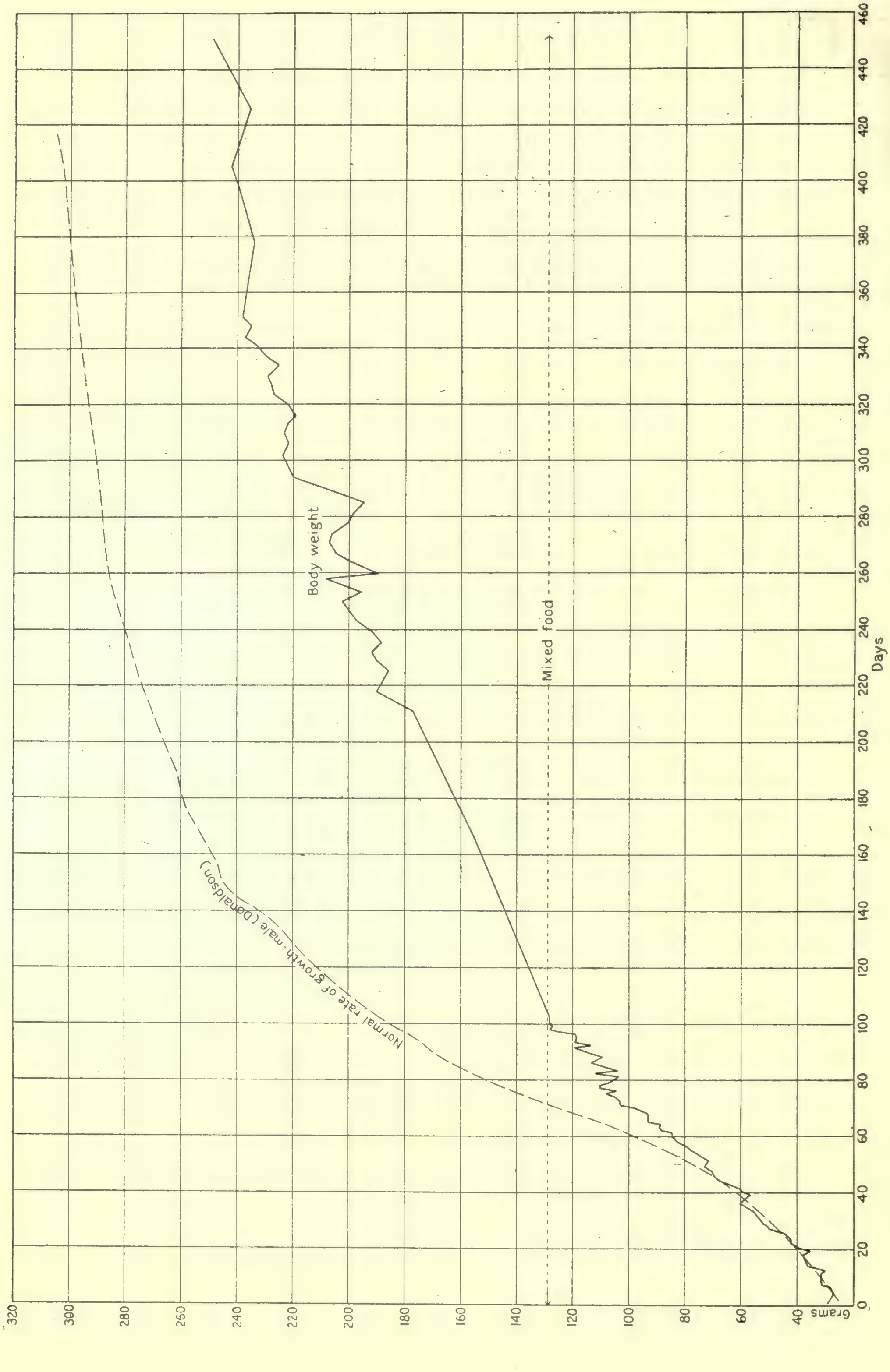


Chart XXII shows average normal rates of growth of male and female white rats according to Donaldson and to Osborne and Mendel. In our experience the female rat does not attain as large a size as in Donaldson's experiments. The growth curves coincide until the animals reach an age of about 70 days.

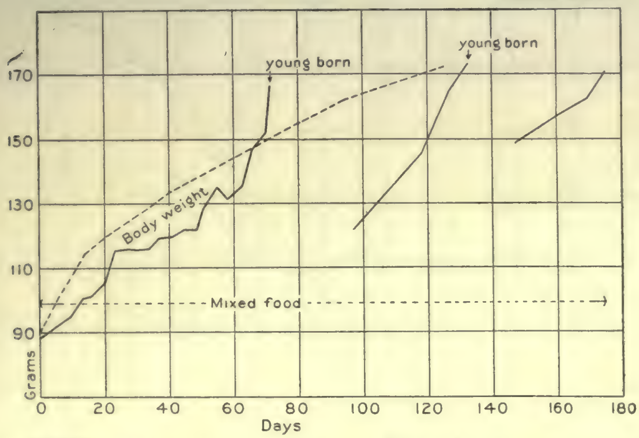
CHART XXIII.



Body weight
Mixed food
Normal rate of growth-male (Donaldson)
Days
Grams
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320

Chart XXIII (rat 48, male) shows the growth of the male rat from early life, under cage conditions adopted for experimental feeding. The animal was fed 452 days on mixed food, consisting of dog-biscuit, sunflower and other seeds, fresh vegetables, and salt.

CHART XXIV.



Charts XXIV (rat 166, female) and XXV (rat 156, female) show the typical growth of female rats, including pregnancy, under cage conditions. The animals were fed on mixed food.

CHART XXV.

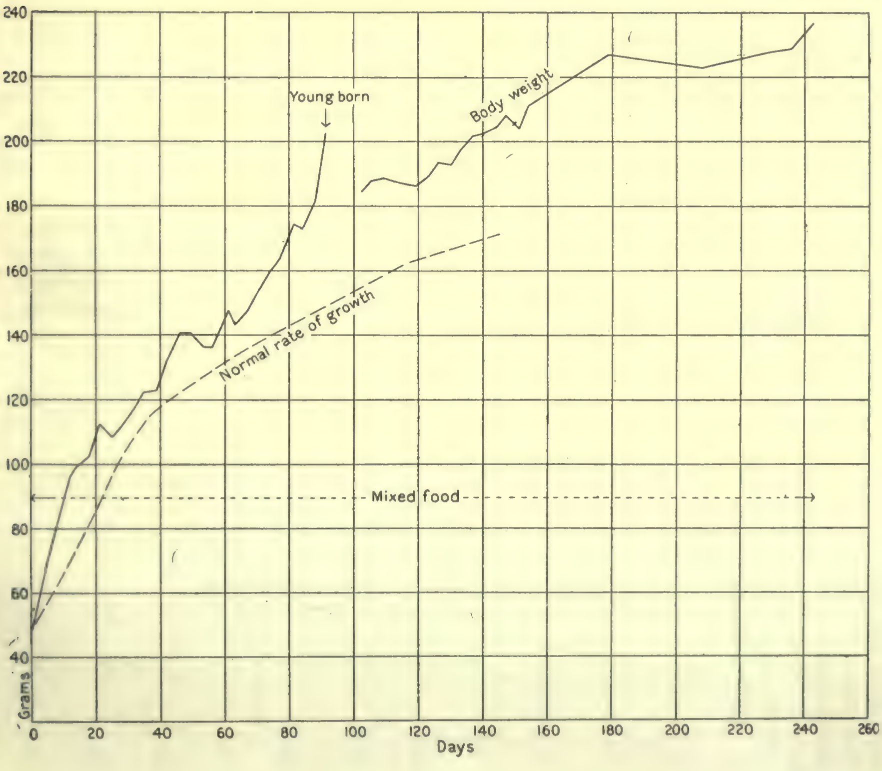


CHART XXVI.

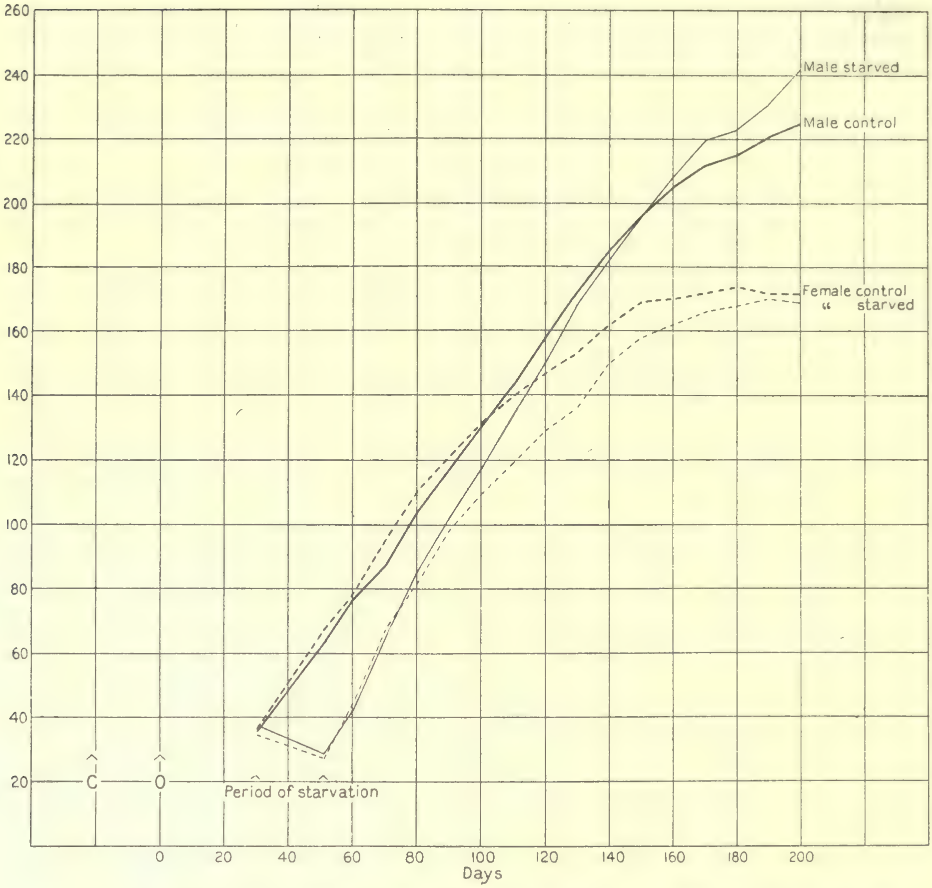


Chart XXVI (curves, from Hatai, American Journal of Physiology, 1907, XVIII, p. 311) shows the body-weights of albino rats at different ages. *C*, conception, and *O*, the date of birth 21 days after conception. An illustration is given of the influence on growth of a period of 21 days of starvation during early life.

CHART XXVII.

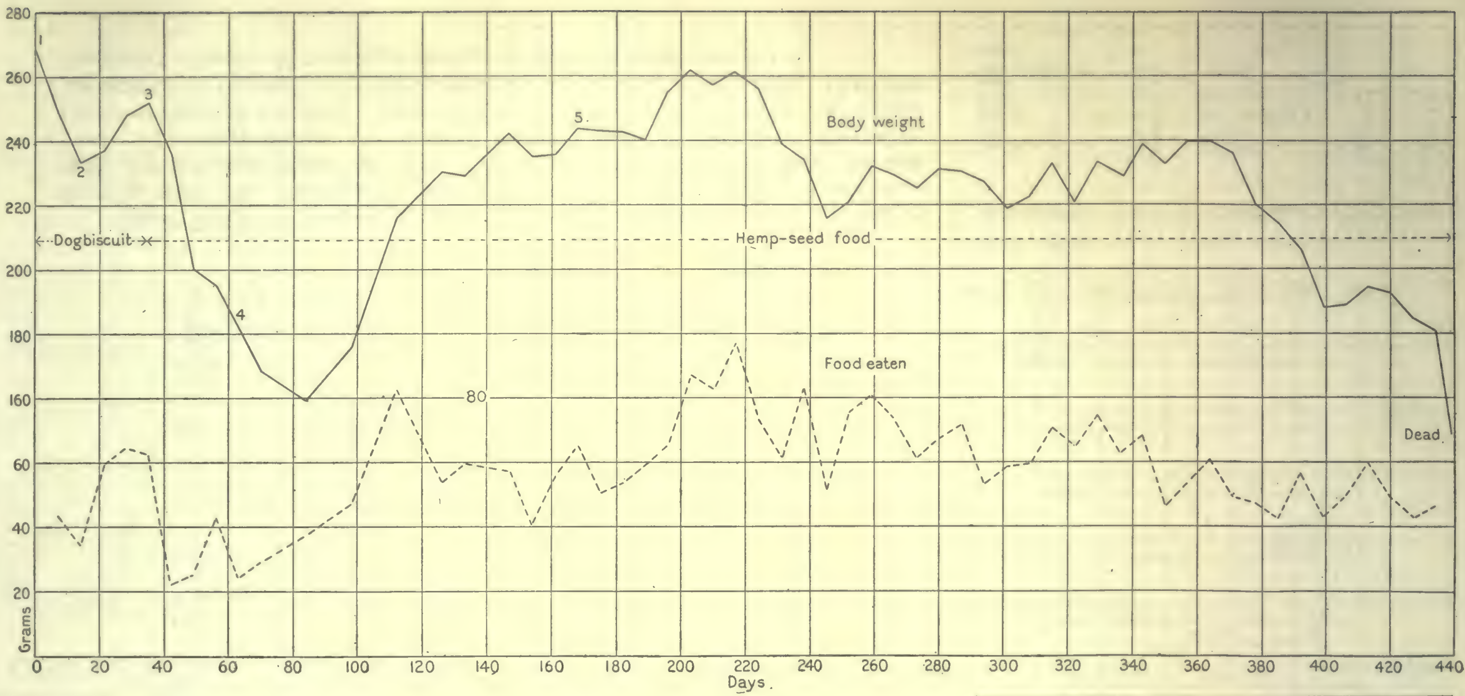


Chart XXVII (rat 18, male) shows the possibility of feeding the same food-mixture (hempseed paste) for a very long period. The diets of this animal for 5 periods were as shown in the table. The hempseed paste was fed for 404 days. Other data will be found on pp. 21–23 in Publication 156, Part I, of the Carnegie Institution of Washington. The food intake was nearly uniform and adequate until near the end of the animal's life. Death was caused by pulmonary disease.

Constituents.	Per. 1.	Per. 2.	Per. 3.	Per. 4.	Per. 5.
	p. ct.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Dog biscuit....	58.4	70.0	0.0	0.0	0.0
Lard..........	41.6	30.0	10.0	10.0	10.0
Hempseed.....	0.0	0.0	46.0	46.0	50.0
Starch........	0.0	0.0	42.0	42.0	38.0
NaCl..........	0.0	0.0	2.0	0.0	0.0
Salt mixture I.	0.0	0.0	0.0	2.0	2.0

CHART XXVIII.

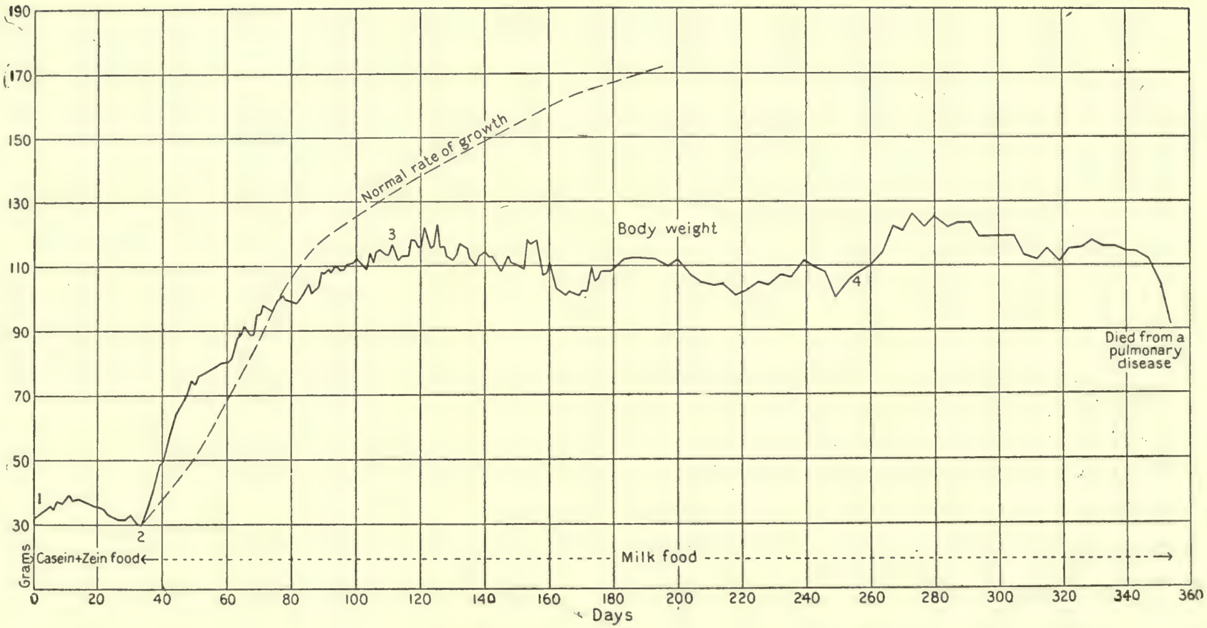


Chart XXVIII (rat 64, female) shows a period of stunting during 33 days in early life, followed by resumption of growth and maintenance on a milk diet for more than 300 days. The animal died of an intercurrent disease. The diets during the several periods were as shown in the table. Observe that the essential change during the long period of milk-paste feeding consisted in modifying the inorganic constituents of the food; also note the entire absence of roughage or agar in the diet after period 1.

Constituents.	Per. 1.	Constituents.	Periods 2 and 4.	Per. 3.
	p. ct.		*p. ct.*	*p. ct.*
Casein	12.0	Trumilk	60.0	60.0
Zein	6.0	Starch	16.7	15.7
Starch	29.5	Salt mixture I	0.0	1.0
Sugar	15.0	Lard	23.3	23.3
Agar	5.0			
Salt mixture I	2.5			
Lard	30.0			

CHART XXIX.

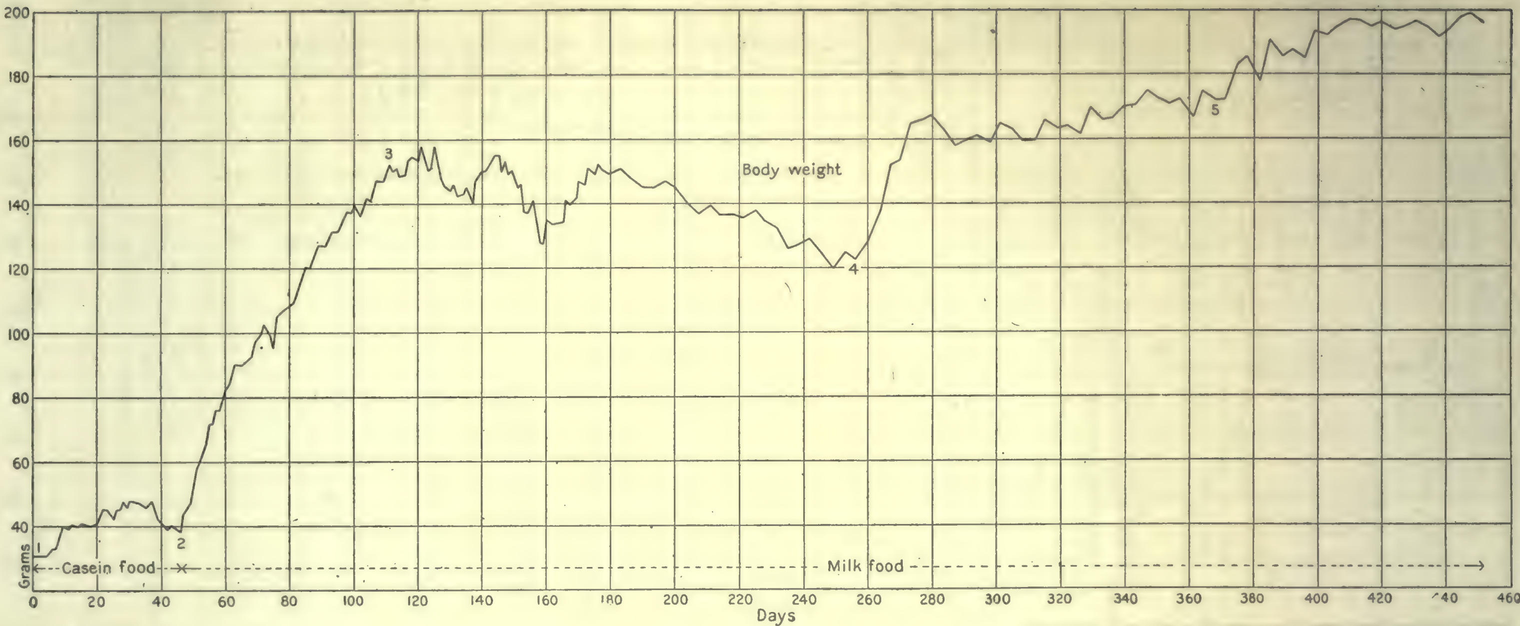


Chart XXIX (rat 51, male) shows early stunted growth on casein (46 days) and recovery on milk diet (406 days). The influence of changes in inorganic salts on nutritive condition is also shown in periods 2 and 4 contrasted with periods 3 and 5. Note the entire absence of roughage or agar in the diet after period 1. The diets were as shown in the table. The efficiency of the milk-paste long continued as a food is apparent, as well as the maintenance of the rat in good health under cage conditions and on a "monotonous" diet.

Constituents.	Per. 1.	Constituents.	Periods 2 and 4.	Periods 3 and 5.
	p. ct.		p. ct.	p. ct.
Casein	18.0	Trumilk	60.0	60.0
Starch	29.5	Starch	16.7	15.7
Sugar	15.0	Salt mixture I.	0.0	1.0
Agar	5.0	Lard	23.3	23.3
Salt mixture I.	2.5			
Lard	30.0			

CHART XXX.

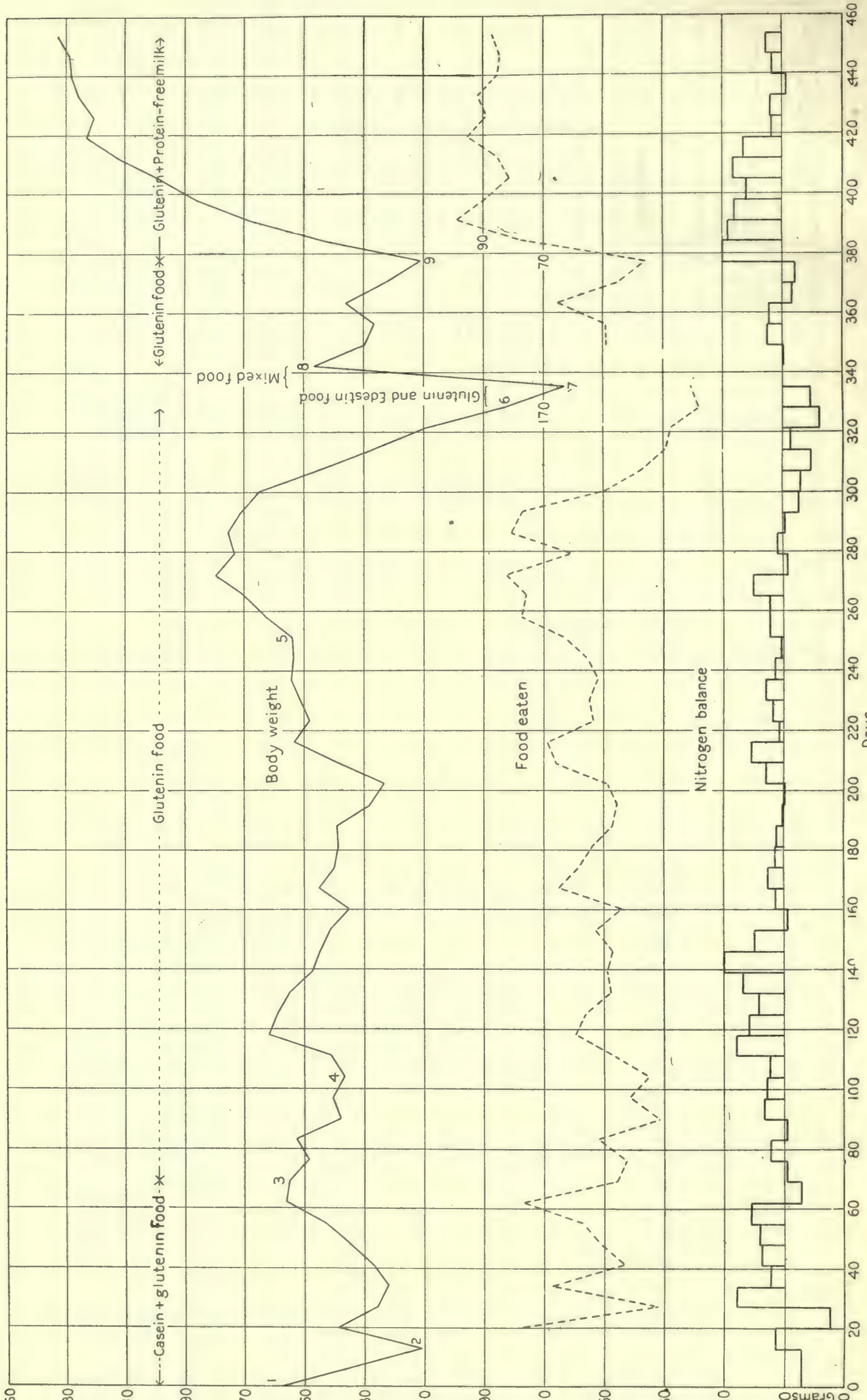

Casein + glutenin food
Glutenin food
Glutenin and Edestin food
Mixed food
Glutenin food
Glutenin + Protein-free milk
Body weight
Food eaten
Nitrogen balance
Days
Grams 0
+0.10

Chart XXX (rat 71, male) shows long-continued feeding of isolated foodstuffs and also long-continued maintenance on glutenin from wheat as the only protein. The history of the animal is on p. 59. The diets were as follows:

Constituents.	Per. 1.	Per. 2.	Per. 3.	Per. 4.	Periods 5 and 8.
	p. ct.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Glutenin	6.0	6.0	16.36	18.0	18.0
Casein	12.0	12.0	0.0	0.0	0.0
Starch	29.5	24.5	22.27	14.5	34.5
Sugar	15.0	15.0	13.63	15.0	20.0
Agar	5.0	5.0	4.54	5.0	5.0
Salt mixture I	2.5	2.5	2.27	2.5	2.5
Lard	30.0	35.0	40.90	45.0	20.0

Constituents.	Per. 6.	Per. 7.	Constituents.	Per. 9.
	p. ct.			*p. ct.*
Glutenin	9.0	Mixed food.	Glutenin	18.0
Edestin	9.0		Protein-free milk	28.2
Starch	33.5		Starch	23.8
Sugar	18.5		Agar	5.0
Agar	5.0		Lard	25.0
Salt mixture I	2.5			
Lard	23.5			

Chart XXX further shows the possibility of maintaining an animal satisfactorily under our cage conditions for 458 days. Attention is particularly directed to period 9, during which the only change in the diet consisted in substituting protein-free milk for some of the non-protein components of the dietary. The lowest line represents the nitrogen balance of the rat.

CHART XXXI.

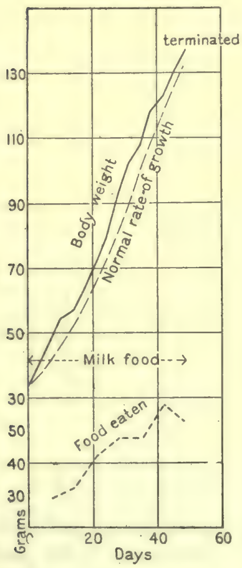


CHART XXXII.

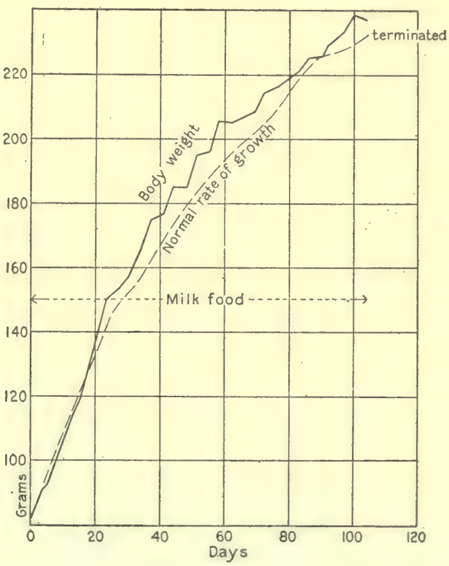


Chart XXXI (rat 222, male) shows early growth curve of male on milk diet, having the following composition: Trumilk, 60.0 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1.0 p. ct.; lard, 23.3 p. ct.

Chart XXXII (rat 195, male) shows normal growth curve of male on milk diet, having the following composition: Trumilk, 60 p. ct.; starch, 16.7 p. ct.; lard, 23.3 p. ct.

CHART XXXIII.

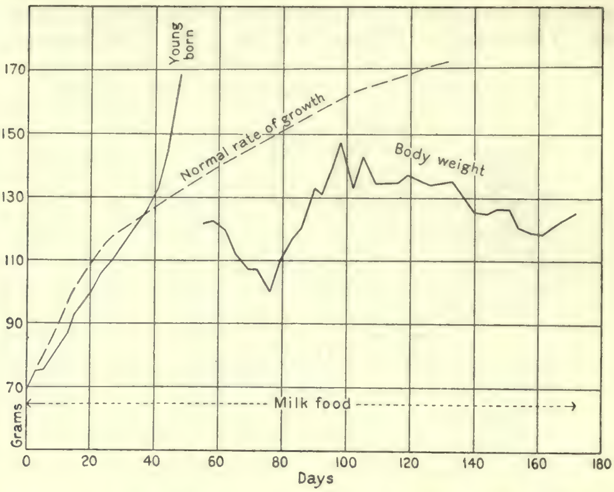


Chart XXXIII (rat 181, female) shows growth and normal pregnancy of female on milk food, consisting of Trumilk, 60 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1.0 p. ct.; lard, 23.3 p. ct.

CHART XXXIV.

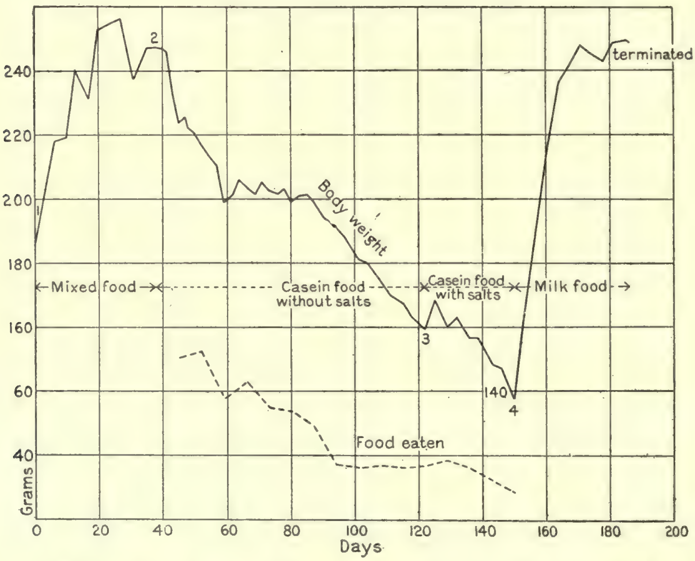


Chart XXXIV (rat 106, male) shows malnutrition induced by lack of inorganic salts in the dietary and subsequent perfect recovery on milk-paste. The diet was mixed food for period 1; for the remaining periods as follows:

Constituents.	Per. 2.	Per. 3.	Constituents.	Per. 4.
	p. ct.	*p. ct.*		*p. ct.*
Casein	18	18.0	Trumilk	60.0
Starch	25 to 32.5	32.5	Starch	15.7
Sugar	17 29.5	21.9	Salt mixture I	1.0
Agar	0 5.0	0.0	Lard	23.3
Salt mixture I	0 0.0	2.6		
Lard	20 35.0	25.0		

CHART XXXV.

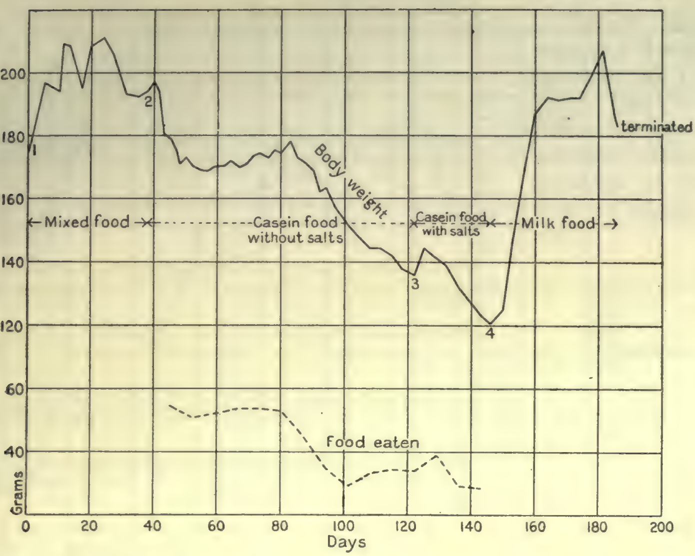


Chart XXXV (rat 110, female) shows malnutrition induced by lack of inorganic salts in the dietary and subsequent perfect recovery on milk-paste. The diet consisted of mixed food for period 1, and as follows for the remaining periods:

Constituents.	Per. 2.	Per. 3.	Constituents.	Per. 4.
	p. ct.	p. ct.		p. ct.
Casein	18	18.0	Trumilk	60.0
Starch	25 to 32.5	32.5	Starch	15.7
Sugar	17 29.5	21.9	Salt mixture I	1.0
Agar	0 5.0	0.0	Lard	23.3
Salt mixture I	0	2.6		
Lard	20 35.0	25.0		

CHART XXXVI.

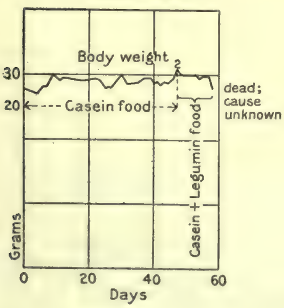


Chart XXXVI (rat 54, male) shows the maintenance for 46 days of a very small rat, without growth, on a diet in which casein formed the sole protein. The composition of the food was as shown herewith:

Constituents.	Per. 1.	Per. 2.
	p. ct.	p. ct.
Casein	18.0	9.0
Pea legumin	0.0	9.0
Starch	29.5	29.5
Sugar	15.0	15.0
Agar	5.0	5.0
Salt mixture I	2.5	2.5
Lard	30.0	30.0

CHART XXXVII.

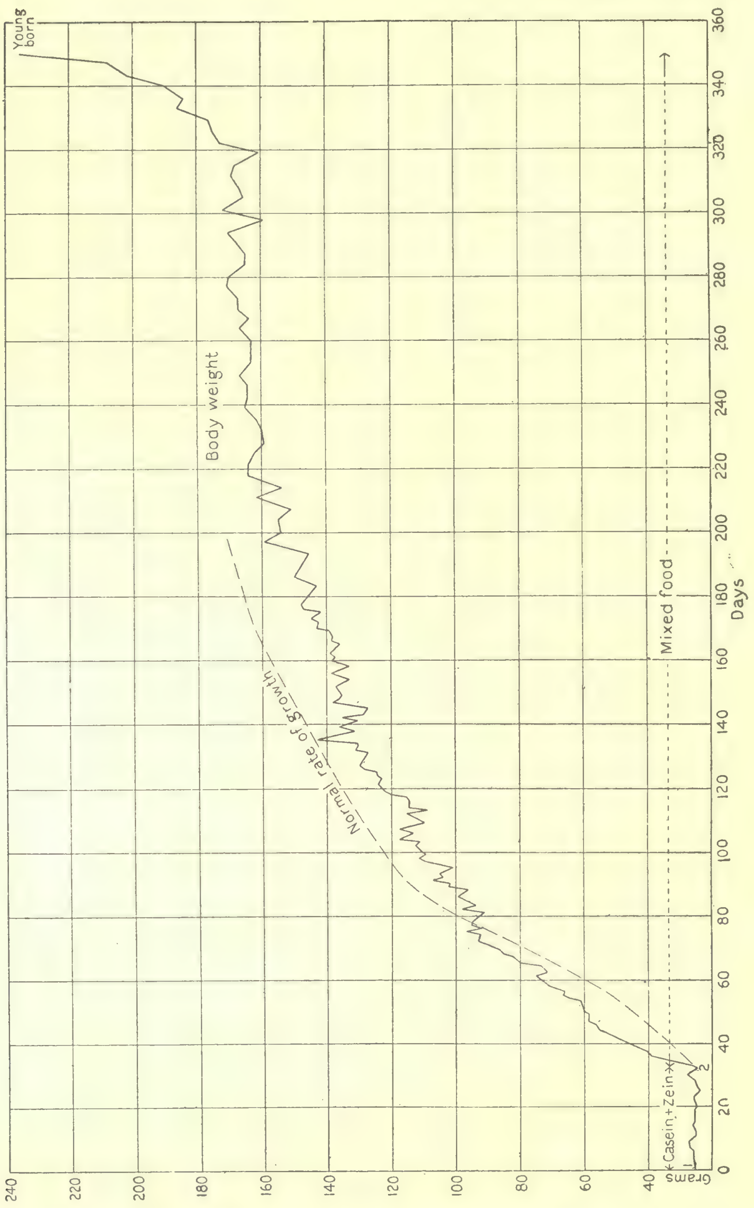


Chart XXXVII (rat 65, female) shows stunting for 33 days during early life, followed by normal growth and pregnancy on mixed food. In addition to the typical growth during 317 days, the curve emphasizes the unaltered "capacity to grow" after stunting by improper diet. The diet during period 1 was as shown herewith.

	p. ct.
Casein	12.0
Zein	6.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I.	2.5
Lard	30.0

CHART XXXVIII.

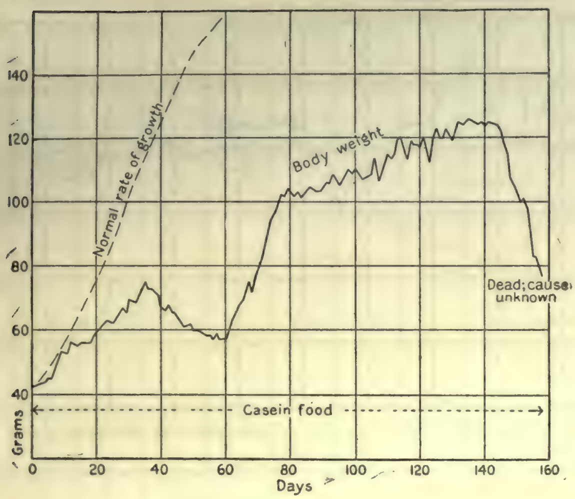


Chart XXXVIII (rat 50) shows maintenance for 158 days on a diet in which casein formed the sole protein. The composition of the food was as shown herewith:

	p. ct.
Casein........	18.0
Starch.......	29.5
Sugar........	15.0
Agar.........	5.0
Salt mixture I.	2.5
Lard.........	30.0

CHART XXXIX.

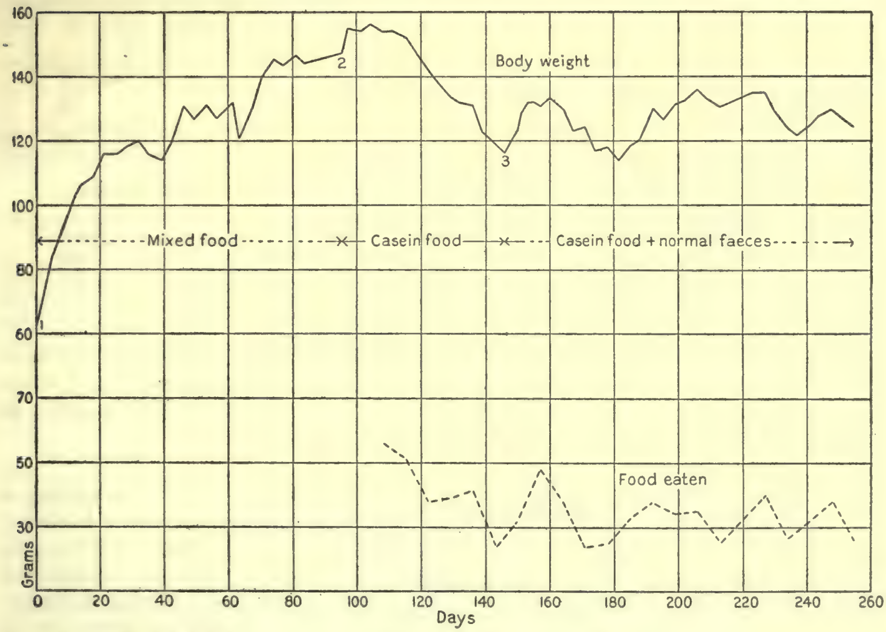


Chart XXXIX (rat 145, female) shows the effect of feeding a diet of isolated food substances in which casein formed the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 3. As shown by the food intake, the favorable effect is not due to an increased consumption of food. The diet during period 1 consisted of mixed food; during periods 2 and 3 as shown in table.

Periods 2 and 3.

	p. ct.	
Casein...........	18.0	....
Starch..........	32.5	....
Sugar...........	21.9 to	26.9
Agar............	0.0	5.0
Salt mixture I....	2.5	2.6
Lard............	20.0	25.0

CHART XL.

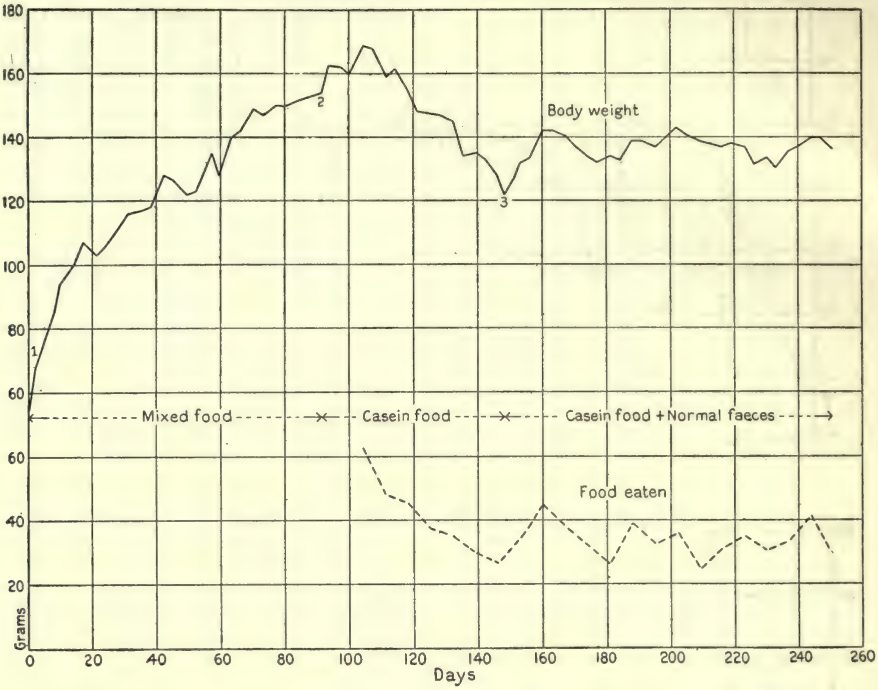


Chart XL (rat 150, female) shows the influence of a diet containing a mixture of isolated foodstuffs in which casein was the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 3. As shown by the food intake the favorable effect is not due to an increased consumption of food. The diet during period 1 consisted of mixed food; during periods 2 and 3 as shown herewith.

Periods 2 and 3.

	p. ct.	
Casein	18.0	....
Starch	32.5	....
Sugar	21.9 to	26.9
Agar	0.0	5.0
Salt mixture I	2.5	2.6
Lard	20.0	25.0

Chart XLI (rat 127, male) shows the influence of a diet containing a mixture of isolated foodstuffs in which casein was the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing for a time the decline in body-weight is shown during period 3. Period 4 shows the favorable nutritive influence of the substitution of protein-free milk for a part of the non-protein constituents of the diet. The diet during period 1 consisted of mixed food. During periods 2, 3, and 4 the composition of the food was as shown herewith.

Constituents.	Periods 2 and 3.		Constituents.	Per. 4.
	p. ct.			*p. ct.*
Casein	18.0	.....	Casein	18.0
Starch	32.5	.....	Protein-free milk	28.2
Sugar	21.9 to	26.9	Starch	23.8
Salt mixture I	2.6	.....	Agar	5.0
Lard	20.0	25.0	Lard	25.0

CHART XLI.

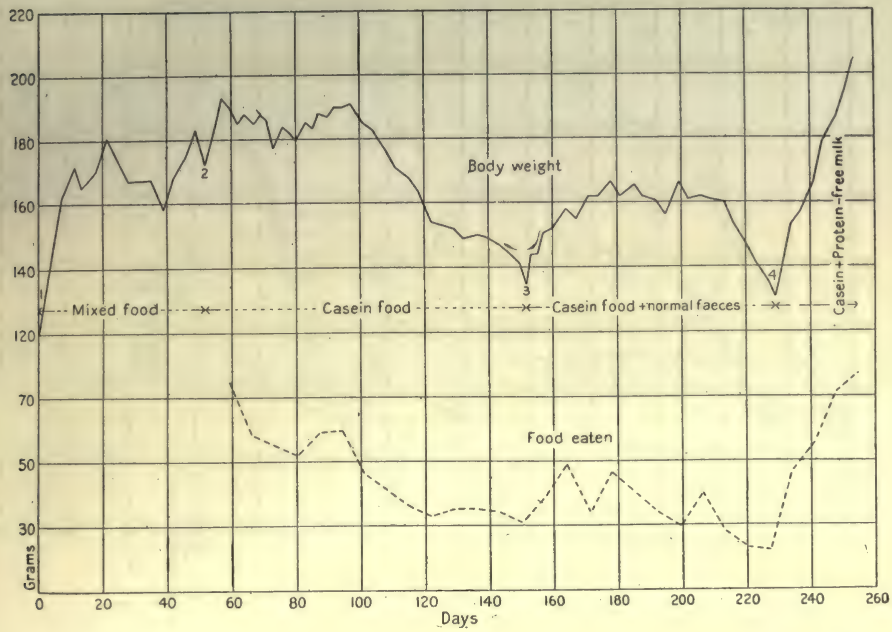


CHART XLII.

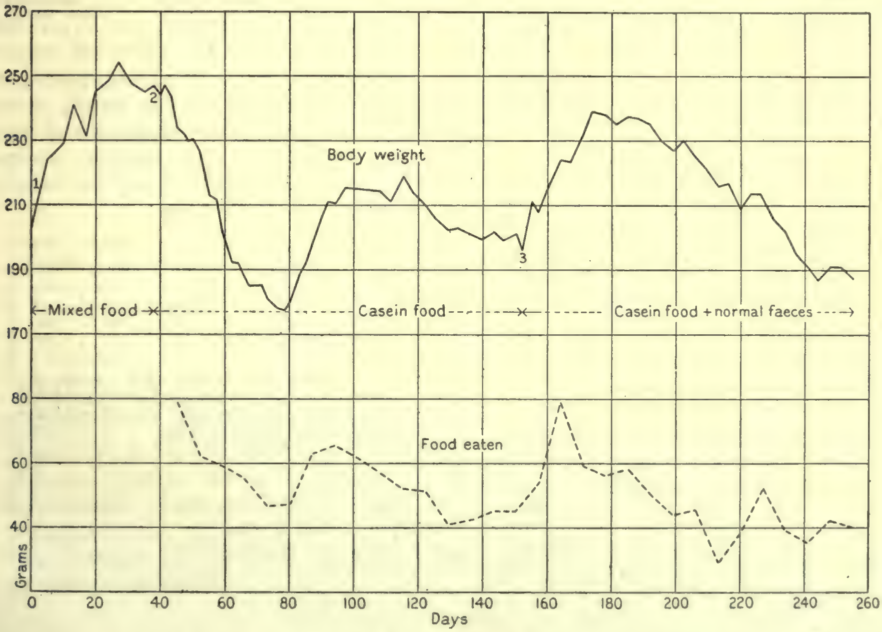


Chart XLII (rat 103, male) shows the influence of a diet of isolated food-stuffs containing casein as the sole protein. The satisfactory previous nutritive condition of the animal is shown during period 1 on mixed food. Casein feeding was begun with period 2; and the favorable effect of fæces of normally fed animals is shown during period 3. The composition of the food in periods 2 and 3 was as shown in table.

	p. ct.	
Casein	18.00	.....
Starch	25.00 to	32.50
Sugar	12.87	25.37
Agar	0.00	5.00
Salt mixture	4.13	.....
Lard	20.00	35.00

The salt mixture, which was prepared for other purposes, consisted of the citrates of calcium, magnesium, sodium, potassium, and iron, and the chlorides of sodium and potassium.

CHART XLIII.

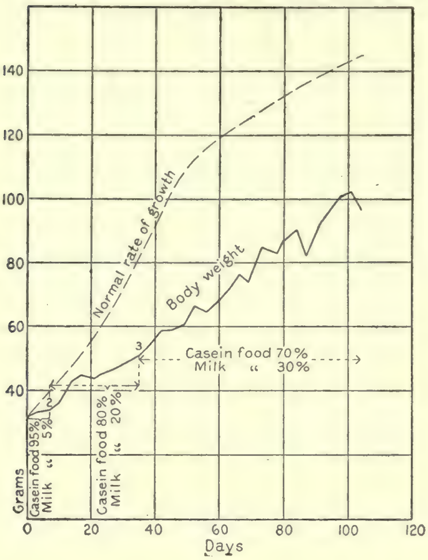


CHART XLIV.

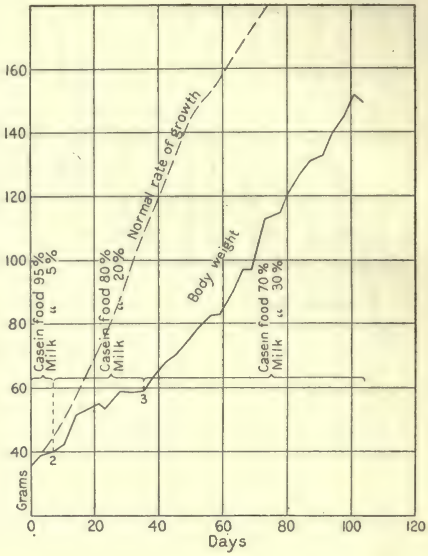
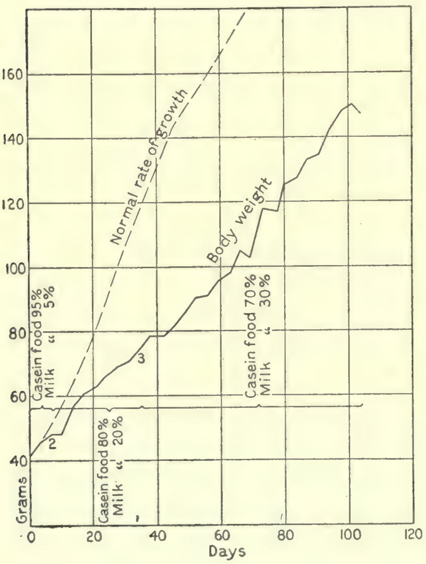


CHART XLV.



Charts XLIII (rat 231, female), XLIV (rat 230, male), and XLV (rat 223, male) show the effect of successive additions of increasing quantities of milk powder to the usual casein diet. The smaller quantities of milk are insufficient to induce normal growth. The diet during the several periods was as follows:

Constituents.	Per. 1.	Per. 2.	Per. 3.
	p. ct.	*p. ct.*	*p. ct.*
*Casein food......	95	80	70
†Milk food.......	5	20	30

*Casein food: casein, 18.0; starch, 32.5; sugar 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.

†Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3.

CHART XLVI.

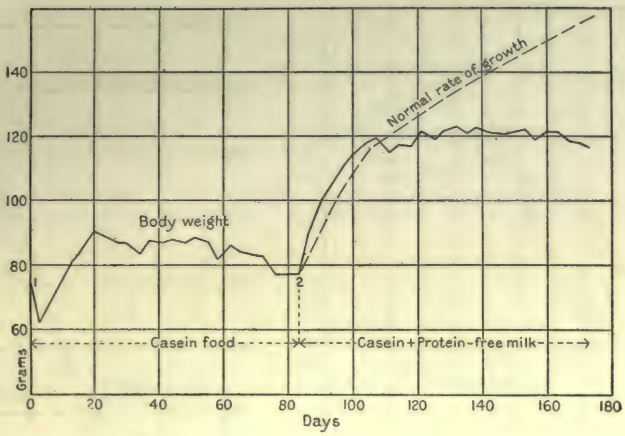
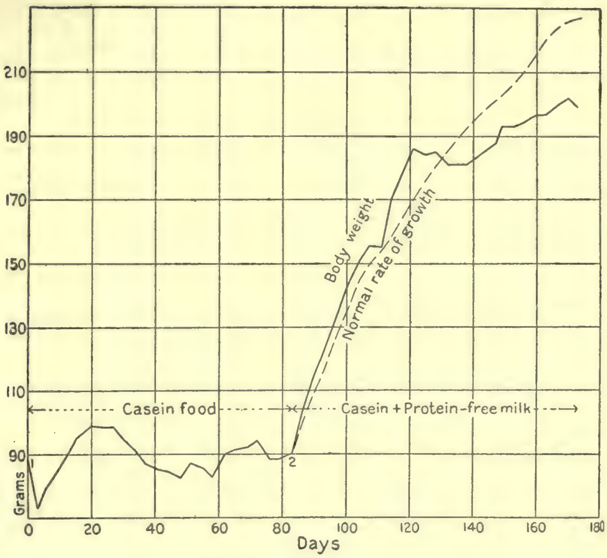


CHART XLVII.



Charts XLVI (rat 177, female) and XLVII (rat 191, male) show maintenance on a diet in which casein formed the sole protein during 83 days followed by growth when protein-free milk was substituted for a part of the non-protein constituents of the diet. The diet was as shown herewith.

Constituents.	Per. 1.	Per. 2.
	p. ct.	*p. ct.*
Casein	18.0	18.0
Protein-free milk	0.0	28.2
Starch	32.5	23.8
Sugar	17.0 to 20.0	0.0
Agar	5.0	5.0
Salt mixture I	2.5	0.0
Lard	22.0 25.0	25.0

CHART XLVIII.

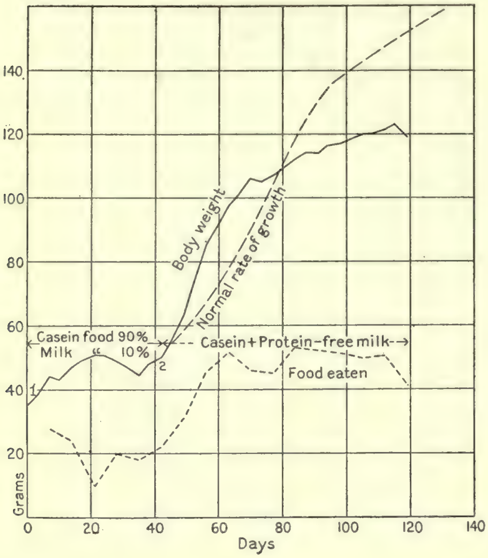
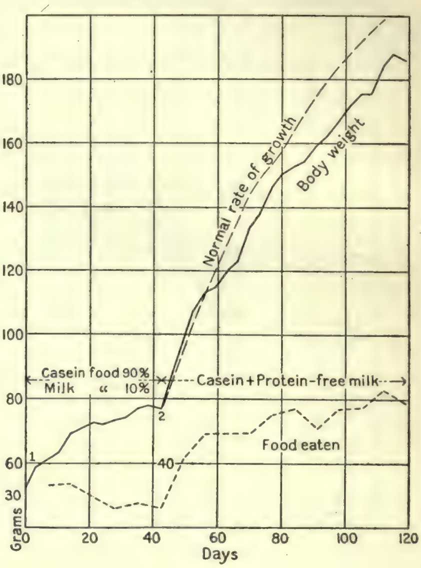


CHART XLIX.



Charts XLVIII (rat 210, female), XLIX (rat 209, male), L (rat 215, male), and LI (rat 216, male) show inadequate growth, during period 1, on the casein food with a small admixture of milk, followed by resumption of growth on a diet containing casein and protein-free milk in a quantity equivalent to that of our milk-paste diet which has proved sufficient to promote normal growth. The composition of the food was as shown in table.

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein food (casein, 18.0; starch, 32.5; sugar, 17.0; agar 5.0; salt mixture I, 2.5; lard, 25.0)..........	90	Casein.......	18.0
		Protein-free milk......	28.2
		Starch......	23.8
		Agar........	5.0
		Lard........	25.0
Milk food, (Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3)..........	10		

CHART L.

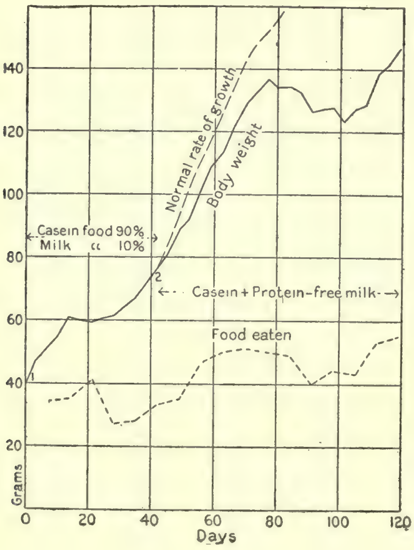


CHART LI.

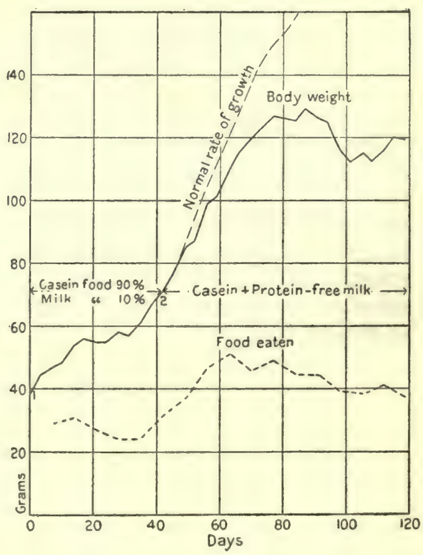


CHART LII.

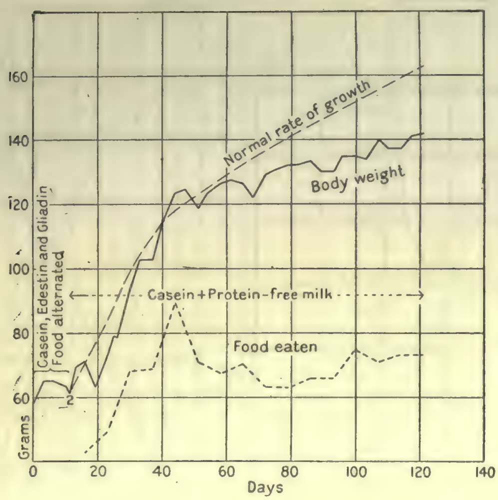
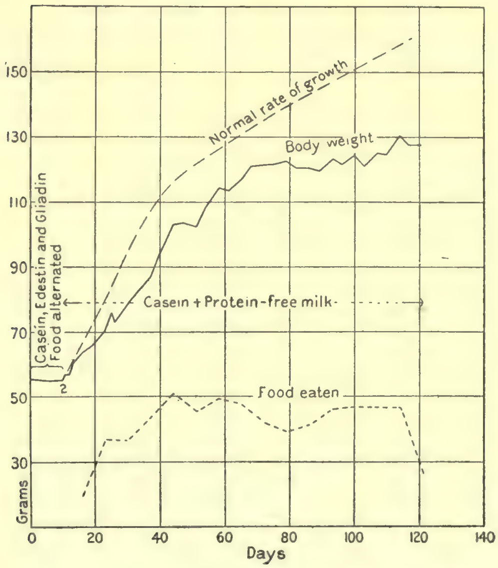


CHART LIII.

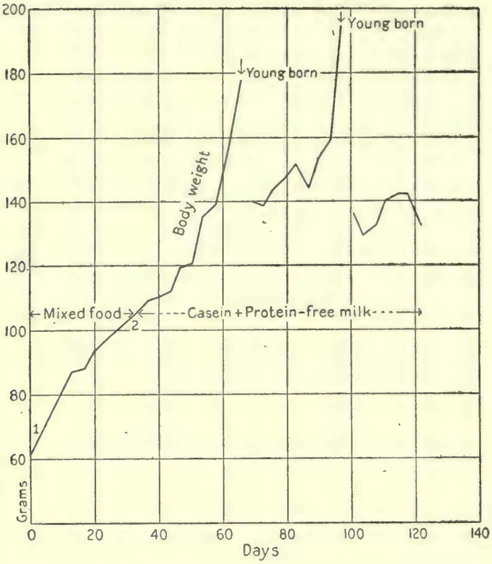


Charts LII (rat 205, female) and LIII (rat 207, female) show initiation of favorable growth when protein-free milk is added to a dietary containing casein as its sole protein in period 2. In the preliminary period an unsuccessful attempt was made to induce growth by feeding different proteins in rotation. The diet was as shown in table.

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein or Edestin or Gliadin	18.0	Casein	18.0
Starch	32.5	Protein-free milk	28.2
Sugar	17.0	Starch	23.8
Agar	5.0	Agar	5.0
Salt mixture I	2.5	Lard	25.0
Lard	25.0		

CHART LV.

CHART LIV.



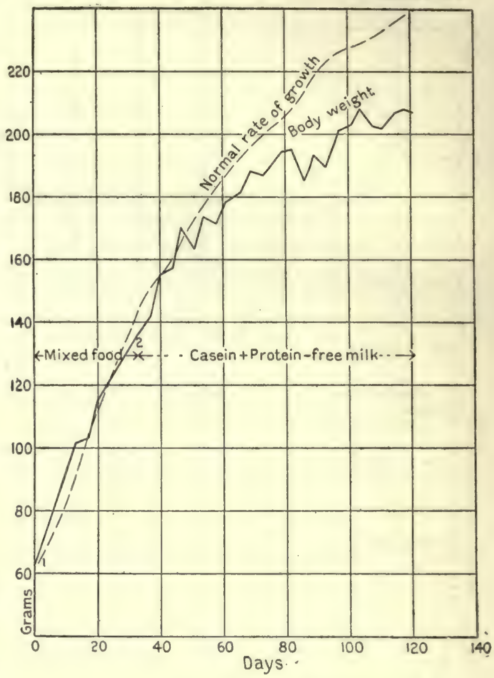


Chart LIV (rat 204, female) shows uninterrupted growth when a diet of isolated food-stuffs containing casein as its sole protein was substituted for mixed food. The requisite inorganic salts were furnished in the added protein-free milk. The experiment is of exceptional interest inasmuch as the animal successfully passed through two periods of pregnancy on a purine-free food containing a single protein. This obviously affords a method of studying various synthetic processes in the animal body. The diet during period 1 consisted of mixed food. During period 2 as shown herewith.

Period 2.	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

Chart LV (rat 203, male) shows uninterrupted growth when a diet of isolated foodstuffs containing casein as its sole protein was substituted for mixed food. The requisite inorganic salts were furnished in the added protein-free milk. The diet during period 1 consisted of mixed food; during period 2, as shown herewith.

Period 2.	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LVI.

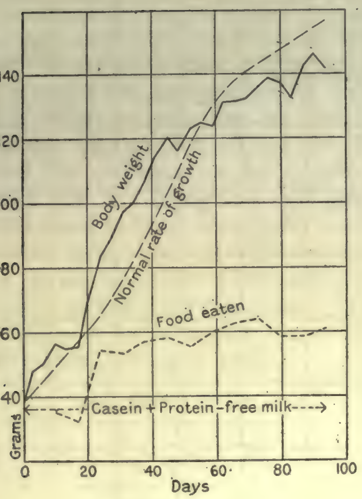


CHART LVII.

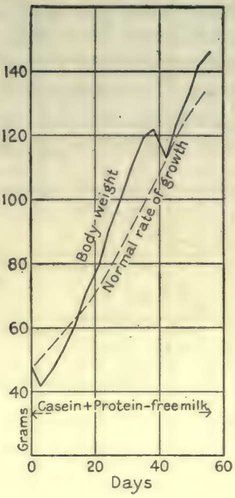


CHART LVIII.

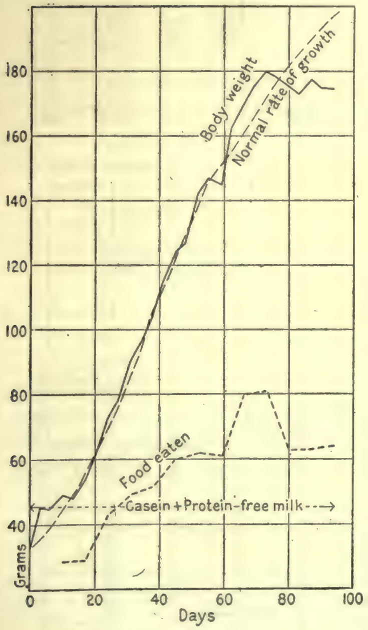


CHART LIX.

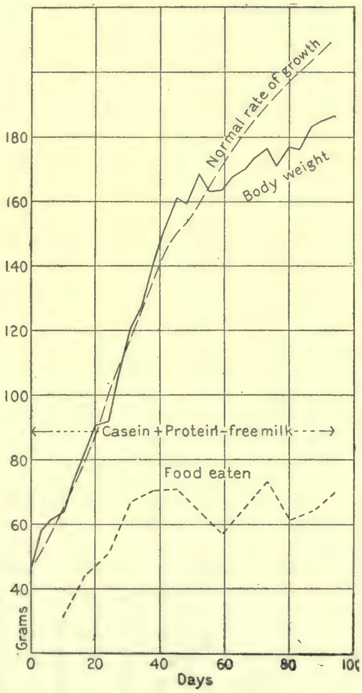
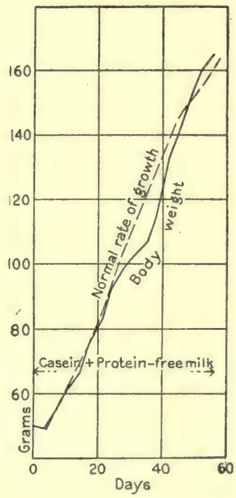


CHART LX.

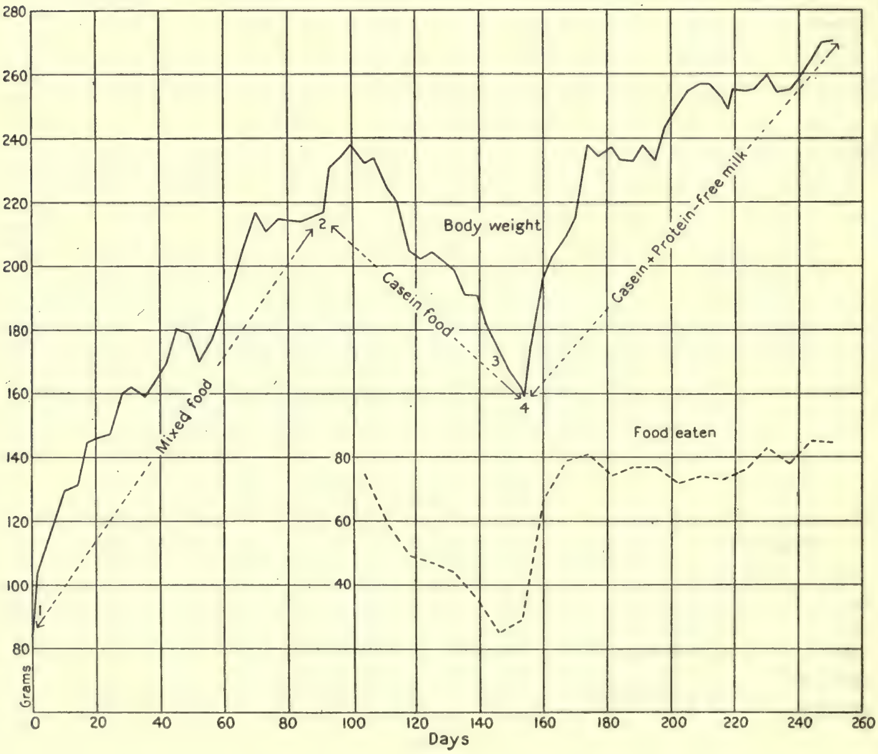


Charts LVI (rat 238, female), LVII (rat 269, female), LVIII (rat 247, male), LIX (rat 252, male), and LX (rat 268, male) show normal growth on a diet containing a single protein, casein. The requisite inorganic salts were furnished in the added protein-free milk. This experiment illustrates artificial nutrition with isolated food-substances from a very early period of life. The diet was as shown herewith.

	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXI.

CHART LXII.



Charts LXI (rat 141, female) and LXII (rat 139, male) show recovery of animals maintained on a diet containing casein as the sole protein. The preliminary nutritive condition of the rats is shown to be satisfactory in period 1 on a mixed diet. The ultimate decline on the casein diet during period 2 could not be checked by increasing the content of casein during period 3. This shows that the nutritive failure of the animals was not attributable to the protein *per se*. Speedy recuperation and maintenance attended the substitution of protein-free milk for the inorganic salt mixture contained in food previously used. Note the influence of this dietary change on the appetite of the animals. In period 1 mixed food was used. The composition of food, during the other periods was as shown in table.

Constituents.	Per. 2.		Per. 3.	Per. 4.
	p. ct.		*p. ct.*	*p. ct.*
Casein	18.0	.....	36.0	18.0
Protein-free milk	0.0	.....	0.0	28.2
Starch	32.5	.....	22.5	23.8
Sugar	21.9 to	26.9	13.9	0.0
Agar	0.0	5.0	0.0	5.0
Salt mixture I	2.5	2.6	2.6	0.0
Lard	20.0	25.0	25.0	25.0

CHART LXIII.

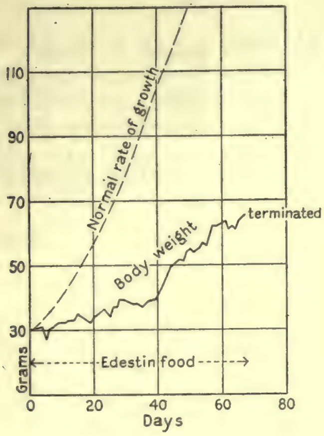
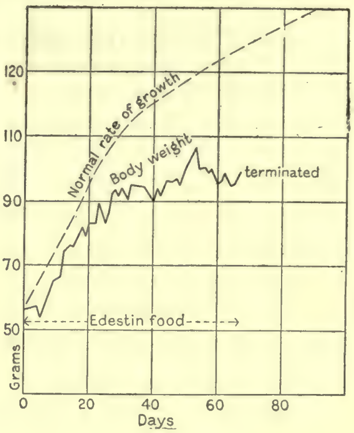


CHART LXIV.



Charts LXIII (rat 60, male) and LXIV (rat 58, female) show maintenance and slight growth of a rat on a diet in which edestin constituted the sole protein for 67 days. The experiment was terminated because of the death of another animal, which was found partly eaten, in the same cage. The diet was as shown herewith.

	p. ct.
Edestin	18.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I.	2.5
Lard	30.0

CHART LXV.

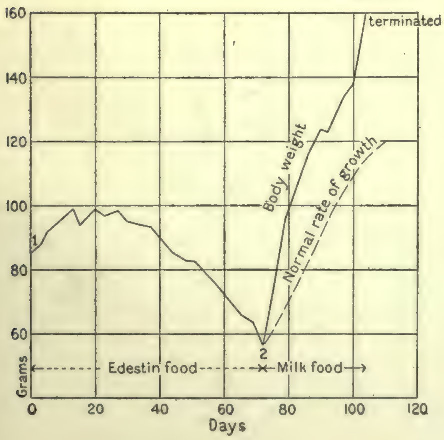
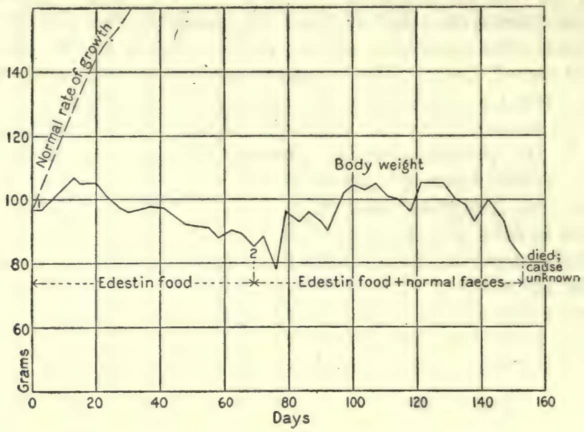


Chart LXV (rat 189, female) shows failure of rat to grow or be maintained on a diet containing edestin as the sole protein during 72 days (period 1). There is no loss of capacity to grow, as will be seen by the curve of growth on the milk diet in period 2, 32 days. The diet consisted of—

Period 1.	*p. ct.*
Edestin	18.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I	2.5
Lard	30.0
Period 2.	
Trumilk	60.0
Starch	15.7
Salt mixture I	1.0
Lard	23.3

CHART LXVI.



Charts LXVI (rat 169, male) and LXVII (rat 190, male) show maintenance on a diet in which edestin formed the sole protein. The influence of fæces of normally fed animals in preventing decline in body-weight for some time is shown during period 2. The fæces were obtained from rats temporarily introduced into the cage each day. The diet is given above.

	p. ct.	
Edestin	18.0	....
Starch	29.5 to	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture, I	2.5	....
Lard	25.0	30.0

CHART LXVII.

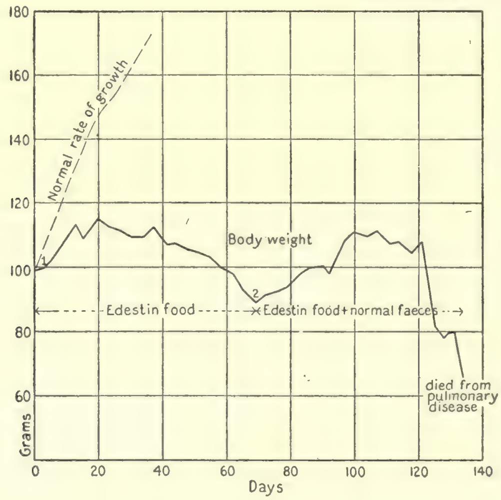
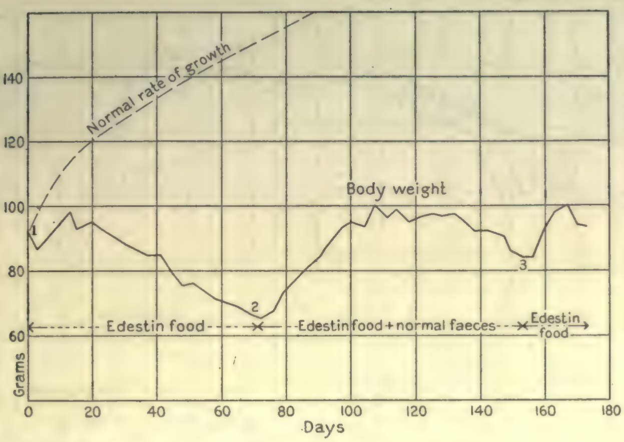


CHART LXVIII.



Charts LXVIII (rat 196, female) and LXIX (rat 193, female) show maintenance on a diet in which edestin formed the sole protein. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 2. The giving of fæces was discontinued during period 3. The fæces were obtained from normally fed rats temporarily introduced into the cage each day. The diet is given above.

	p. ct.	
Edestin	18.0	....
Starch	29.5 to	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART LXIX.

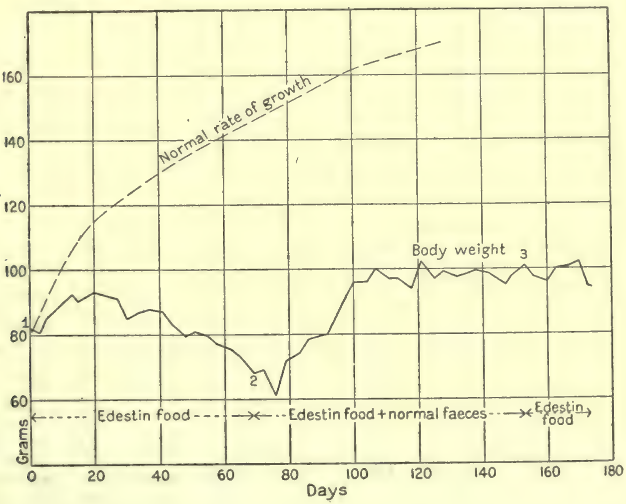


CHART LXX.

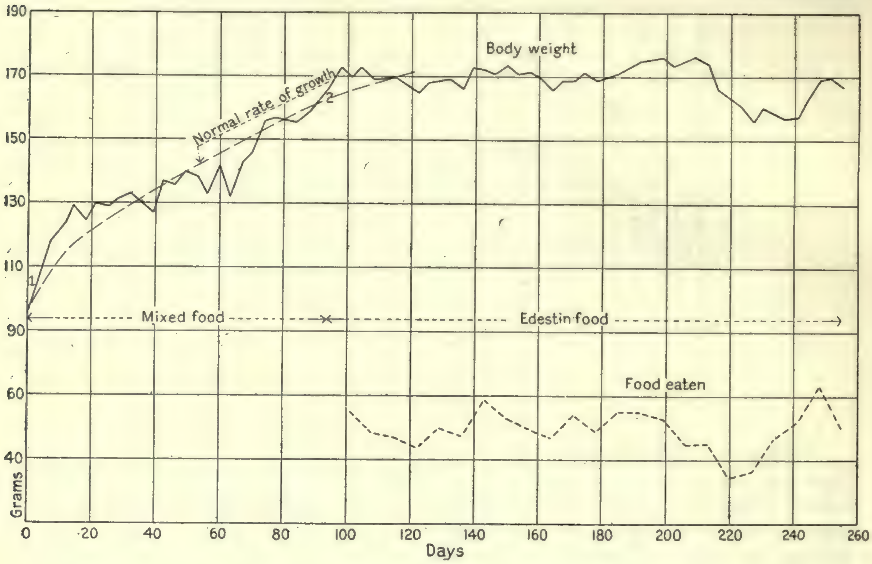


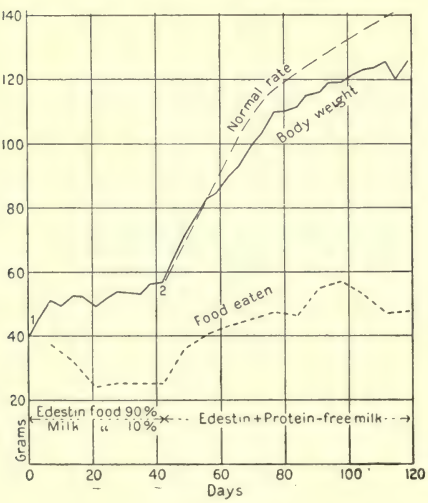
Chart LXX (rat 133, female) shows maintenance on a diet in which edestin was the sole protein during 161 days. Period 1 on a mixed diet shows normal growth. Period 2 is of interest because the food was also purine-free and devoid of organically combined phosphorus. All growth ceased during the edestin feeding (period 2), in contrast with other experiences where protein-free milk was present in the dietary.

Period 2.

	p. ct.	
Edestin	18.0	....
Starch	29.5 *to*	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

Chart LXXI (rat 218, female) shows inadequate growth on a diet of edestin+milk-paste (period 1) followed by growth during period 2, in which the food contained protein-free milk and edestin as its sole protein. In growing to several times its original weight the animal must have synthesized its purine- and phosphorus-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curve. The diet consisted of—

CHART LXXI.



Period 1.	*p. ct.*
Edestin food (edestin, 18.0; starch, 32.5: sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0)	90
Milk food (Trumilk, 60.0; starch 15.7; salt mixture I, 1.0; lard, 23.3.)	10

Period 2.	
Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXXII.

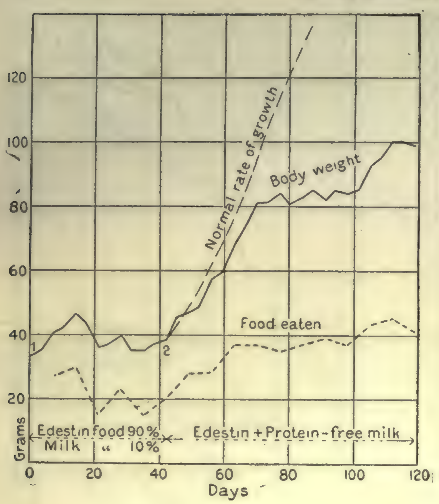


CHART LXXIV.

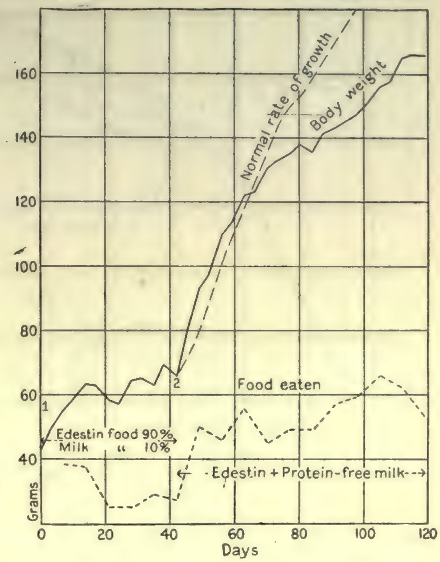
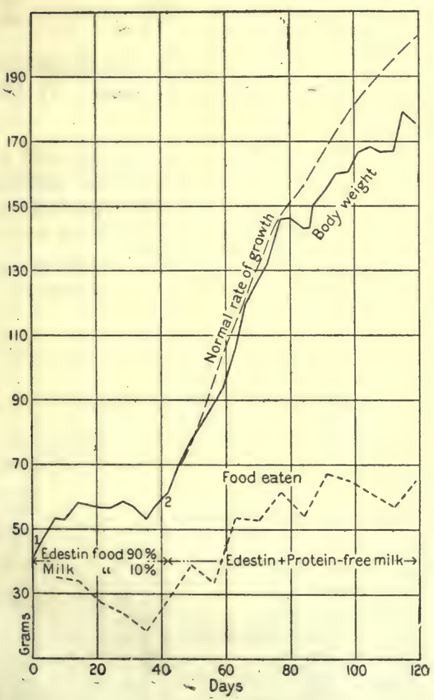


CHART LXXIII.



Charts LXXII (rat 217, male), LXXIII (rat 211, male), and LXXIV (rat 212, male) show inadequate growth on a diet of edestin+milk-paste (period 1) followed by growth during period 2, in which the food contained protein-free milk and edestin as its sole protein. It should be noted that the animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food intake curve. The diet consisted of—

Period 1.	*p. ct.*
Edestin food (edestin, 18.0; starch, 32.5; sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0)	90.0
Milk food (Trumilk, 60.0; starch; 15.7; salt mixture I, 1.0; lard,, 23.3)	10.0

Period 2.	
Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXXV.

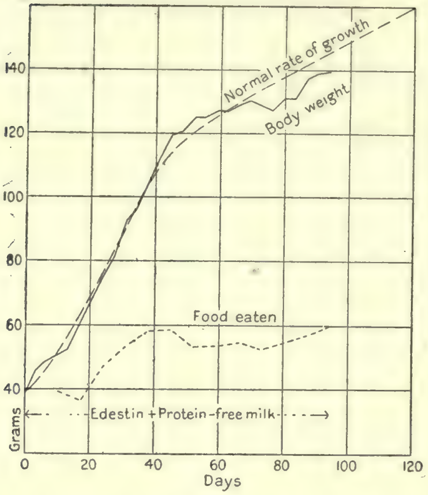
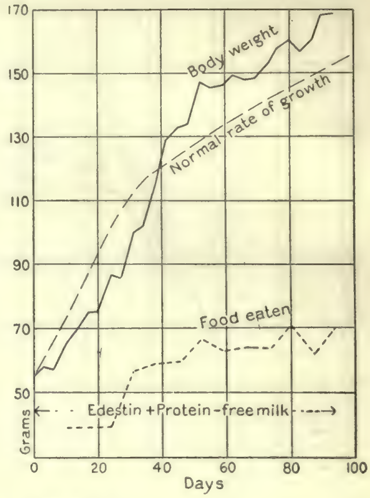


CHART LXXVI.



Charts LXXV (rat 248, female) and LXXVI (rat 253, female) show growth from an early age on a diet containing protein-free milk in which edestin formed the sole protein. It should be noted that the animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curve. The diet was as shown herewith.

	p. ct.
Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

Chart LXXVII (rat 114, male) shows the failure of edestin (period 2) to maintain previous satisfactory nutritive condition of the animal during period 1, on mixed food, even after adding fæces to the diet (period 3). Immediate improvement and satisfactory nutritive condition followed addition of protein-free milk to edestin food (period 4). The diet consisted of mixed food for period 1, and for periods 2, 3, and 4 was as shown in table.

Constituents.	Periods 2 and 3.		Constituents.	Per. 4.
	p. ct.			*p. ct.*
Edestin	18.0	.....	Edestin	18.0
Starch	29.5 to	32.5	Protein-free milk	28.2
Sugar	15.0	17.0	Starch	23.8
Agar	5.0	.....	Agar	5.0
Salt mixture I	2.5	.....	Lard	25.0
Lard	25.0	30.0		

Chart LXXVIII (rat 140, female) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. The diet consisted of mixed food for period 1, and for periods 2 and 3 it was as shown in table.

Constituents.	Per. 2.		Per. 3.
	p. ct.		*p. ct.*
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to	32.5	23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXVII.

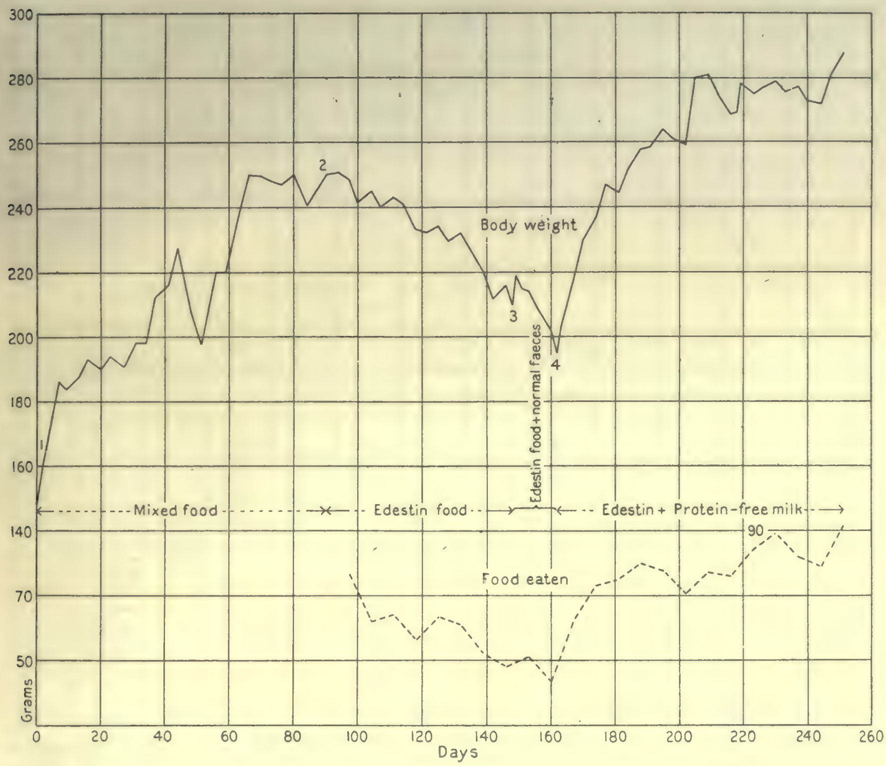


CHART LXXVIII.

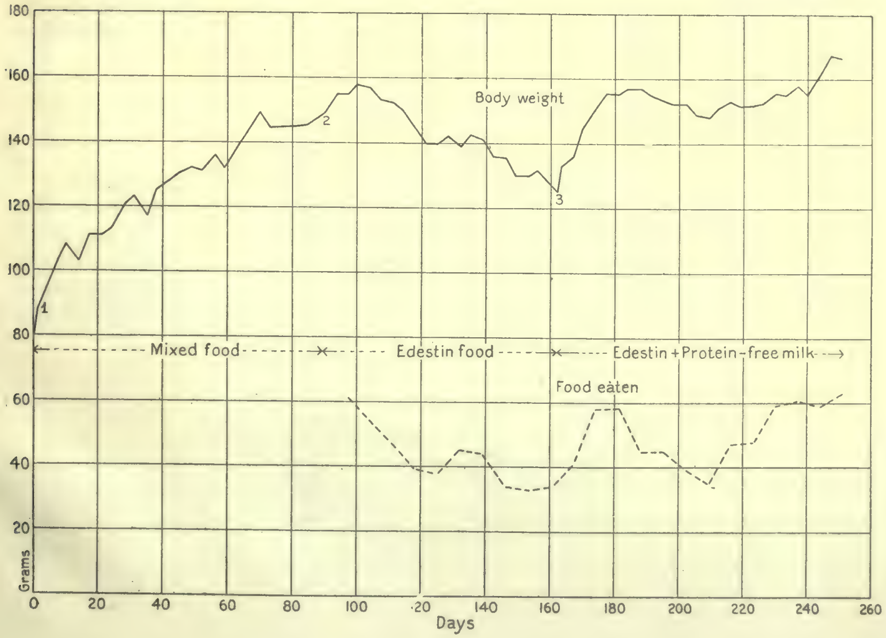


CHART LXXIX.

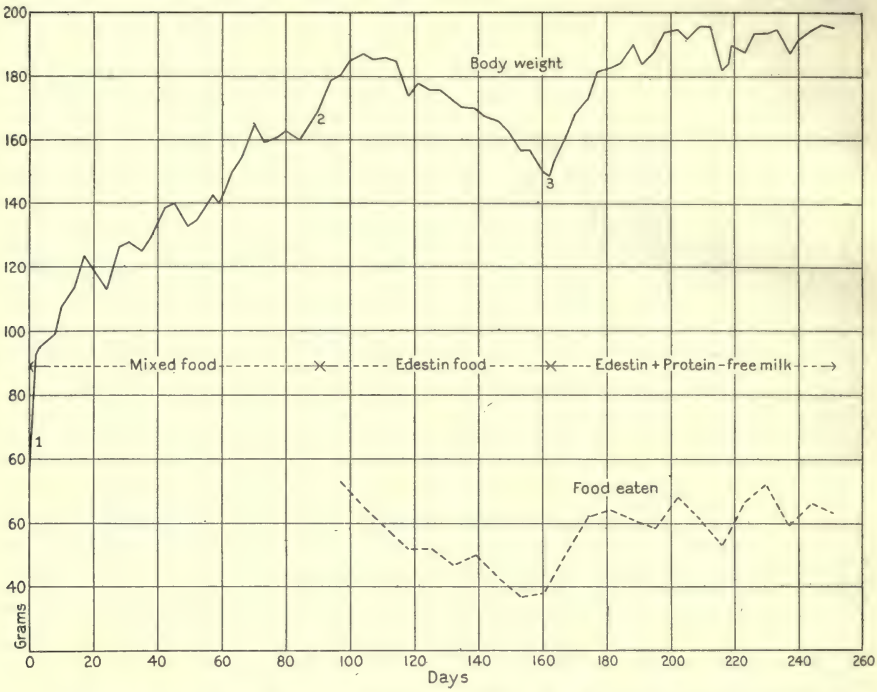


Chart LXXIX (rat 152, female) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. The diet consisted of mixed food for period 1 and for periods 2 and 3 was as follows:

Constituents.	Per. 2.		Per. 3.
	p. ct.		*p. ct.*
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to	32.5	23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXX.

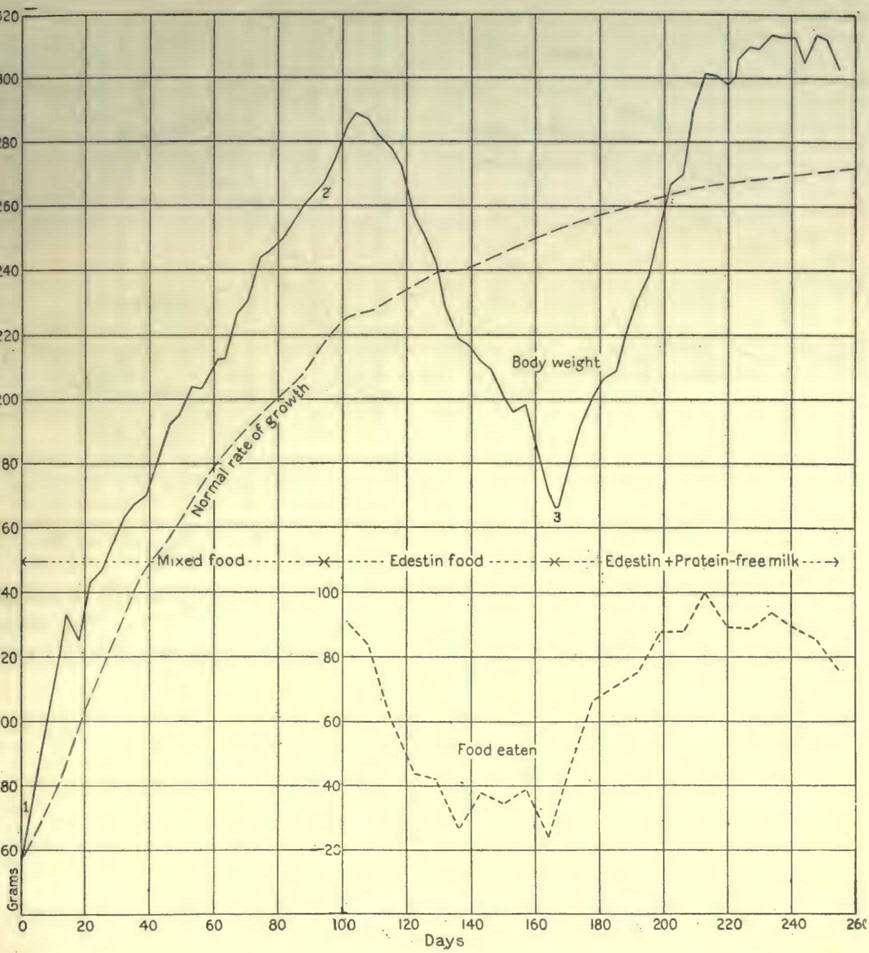


Chart LXXX (rat 148, male) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. Note the influence of changes in diet on the food consumption. The diet consisted of mixed food for period 1, and for periods 2 and 3 was as follows:

Constituents.	Per. 2.		Per. 3.
	p. ct.		*p. ct.*
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to	32.5	23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXXI.

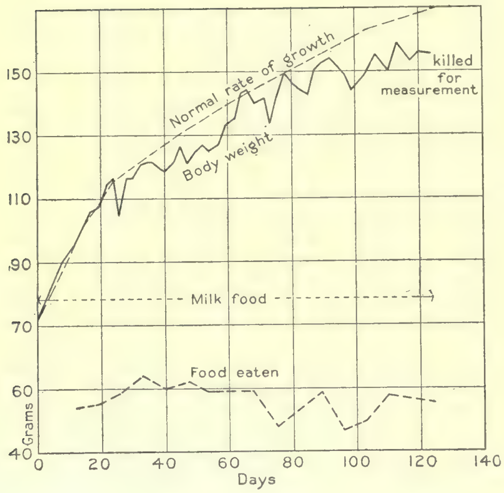
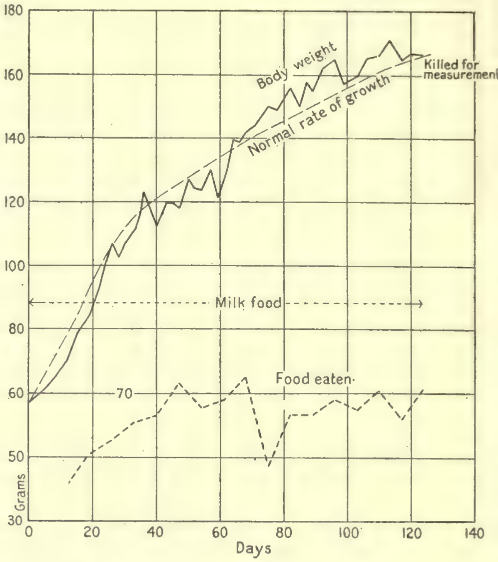


CHART LXXXII.



Charts LXXXI (rat 96, female) and LXXXII (rat 97, female). Control animals for the glutenin dwarfs, Charts LXXXIV–LXXXVI. For other data see page 73. The diet consisted of Trumilk, 60 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1 p. ct., lard, 23.3 p. ct.

CHART LXXXIII.

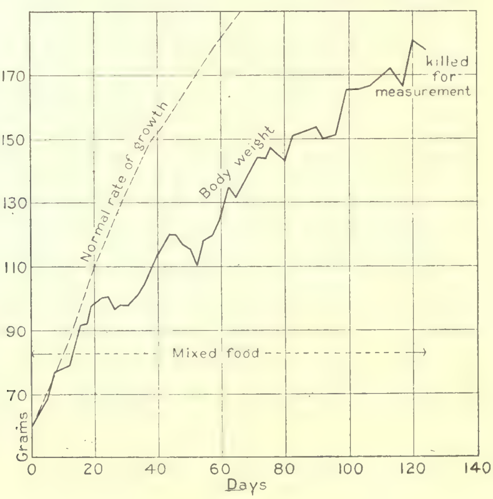


Chart LXXXIII (rat 99, male). Control animal for the glutenin dwarfs, Charts LXXXIV–LXXXVI. For other data see page 73. The diet consisted of mixed food.

CHART LXXXIV.

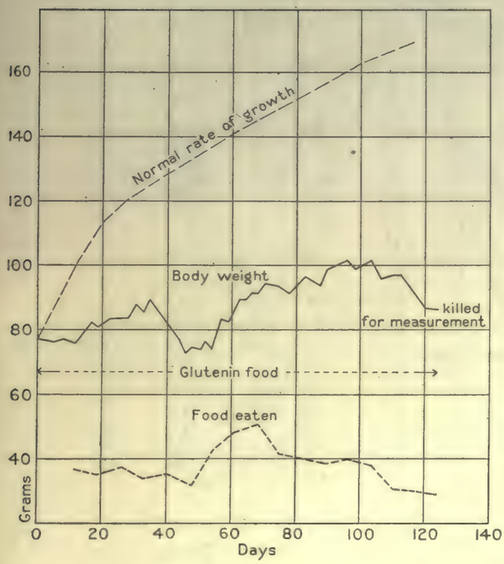


CHART LXXXV.

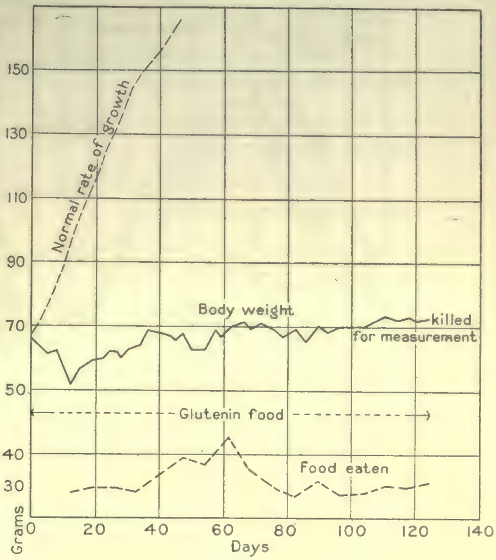
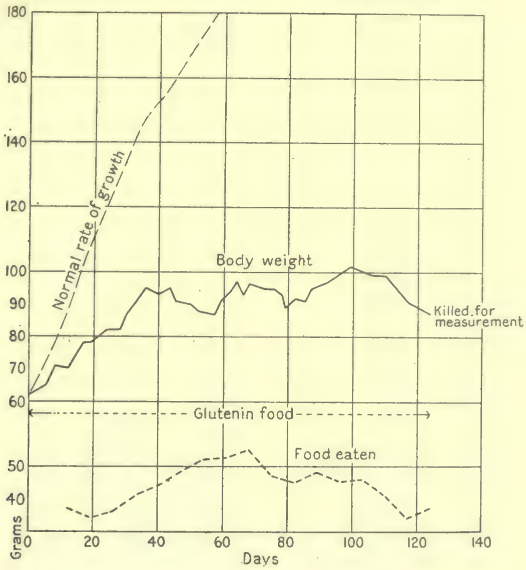


CHART LXXXVI.



Charts LXXXIV (rat 100, female), LXXXV (rat 101, male), and LXXXVI (rat 102, male). These animals, from the same family as the control rats, Charts LXXXI–LXXXIII, were maintained on a diet of glutenin from wheat 124 days, when they were killed for measurement. The chart illustrates maintenance without appreciable growth. For other data see page 73. The diet was as shown herewith.

	p. ct.	
Glutenin	18.0	....
Starch	14.5 to	34.5
Sugar	15.0	20.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	20.0	45.0

CHART LXXXVII.

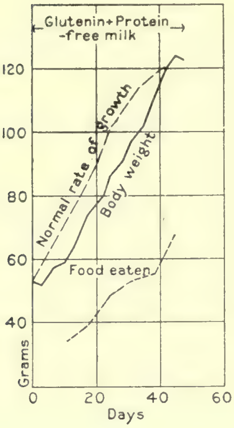


CHART LXXXVIII.

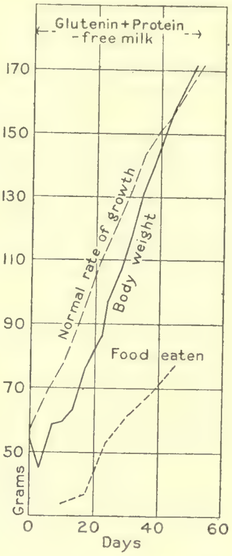
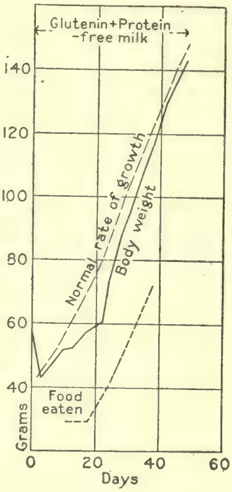


CHART LXXXIX.



Charts LXXXVII (rat 293, female), LXXXVIII (rat 284, male), and LXXXIX (rat 279, male) show growth from an early age on a diet containing protein-free milk, in which glutenin from wheat formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curves. The diet was as shown in table.

	p. ct.
Glutenin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XC.

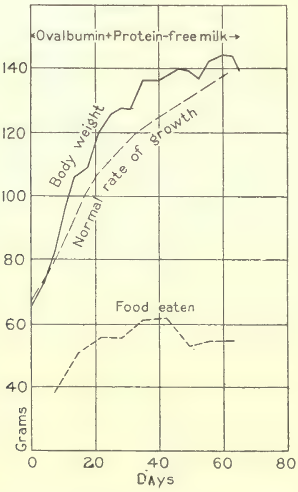
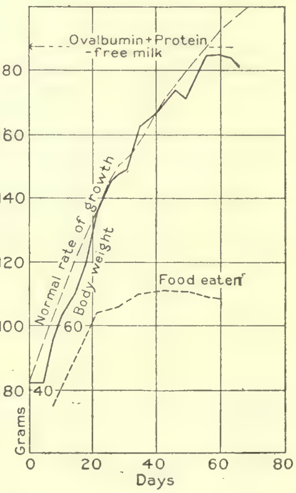


CHART XCI.



Charts XC (rat 258, female) and XCI (rat 250, male) show growth from an early age on a diet containing protein-free milk, in which ovalbumin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curves. The diet was as shown above.

	p. ct.
Ovalbumin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XCII.

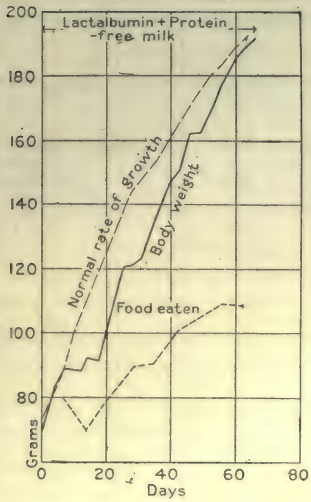
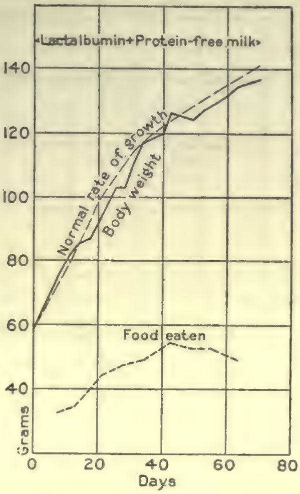


CHART XCIII.



Charts XCII (rat 251, male) and XCIII (rat 259, female) show growth from an early age on a diet containing protein-free milk, in which lactalbumin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curves. The diet was as shown herewith.

	p. ct.	
Lactalbumin	18.0	....
Protein-free milk	28.2	....
Starch	16.8 to	18.8
Agar	5.0	....
Lard	30.0	32.0

CHART XCIV.

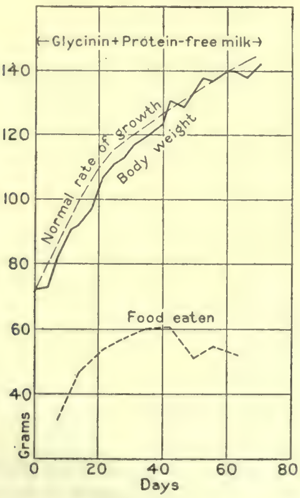
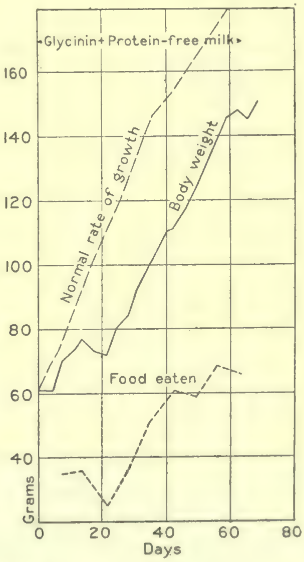


CHART XCV.



Charts XCIV (rat 257, female) and XCV (rat 241, male) show growth from an early age on a diet containing protein-free milk, in which glycinin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curves. The diet was as shown herewith.

	p. ct.
Glycinin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XCVI.

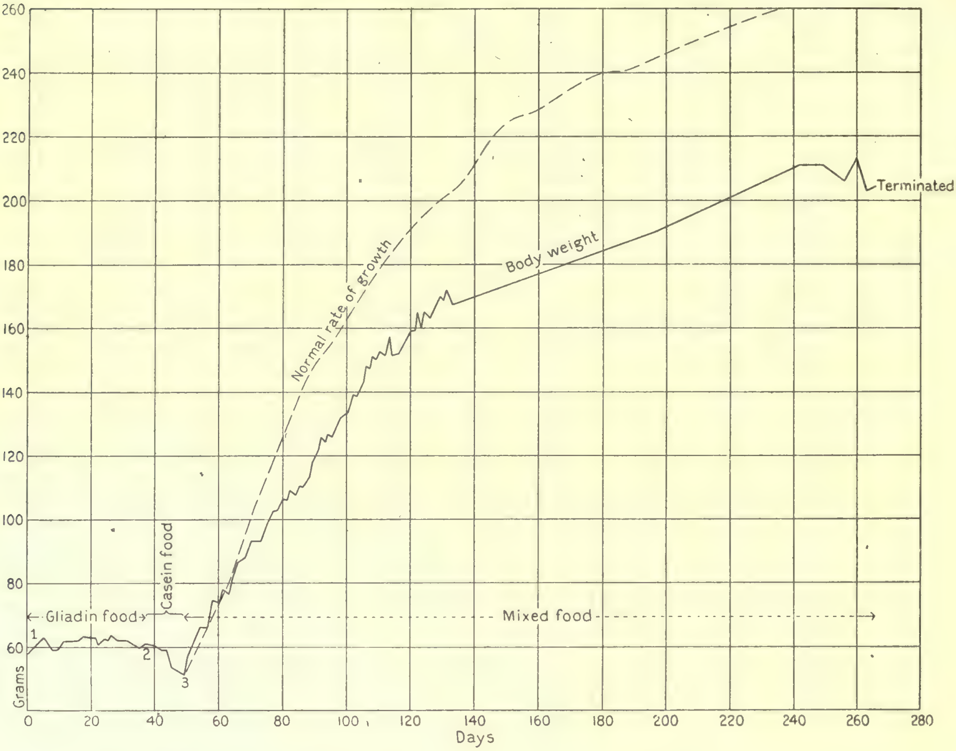


Chart XCVI (rat 36, male) shows the failure of inhibition of growth to check the "capacity to grow." The rat was stunted on gliadin food for 37 days (period 1) and on casein food for 12 days (period 2) and completely recovered growth on mixed diet during 217 days (period 3). The diet for periods 1 and 2 was as follows:

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Gliadin (from wheat)	18.0	Casein	18.0
Starch	29.5	Starch	29.5
Sugar	15.0	Sugar	15.0
Agar	5.0	Agar	5.0
Salt mixture I	2.5	Salt mixture I	2.5
Lard	30.0	Lard	30.0

Chart XCVII (rat 37, male) shows unimpaired capacity for growth on mixed diet and milk diet after an earlier period of stunted growth on gliadin diet for 37 days (period 1) and casein diet for 12 days (period 2). Part of the period of growth was accomplished on milk food, part on mixed food, the change being made at 3 to mixed food, at 4 to milk food, and at 5 to

mixed food again. Note that this has not affected the typical character of the curve of growth. The diet was as follows:

Constituents.	Per. 1.	Constituents.	Per. 2.	Periods 3 and 5.	Constituents.	Per. 4.
	p. ct.		*p. ct.*			*p. ct.*
Gliadin (from wheat)...	18.0	Casein..........	18.0	Mixed food.	Trumilk....	60.0
Starch................	29.5	Starch..........	29.5		Starch.....	16.7
Sugar.................	15.0	Sugar...........	15.0		Lard.......	23.3
Agar..................	5.0	Agar............	5.0			
Salt mixture I.........	2.5	Salt mixture I...	2.5			
Lard..................	30.0	Lard............	30.0			

CHART XCVII.

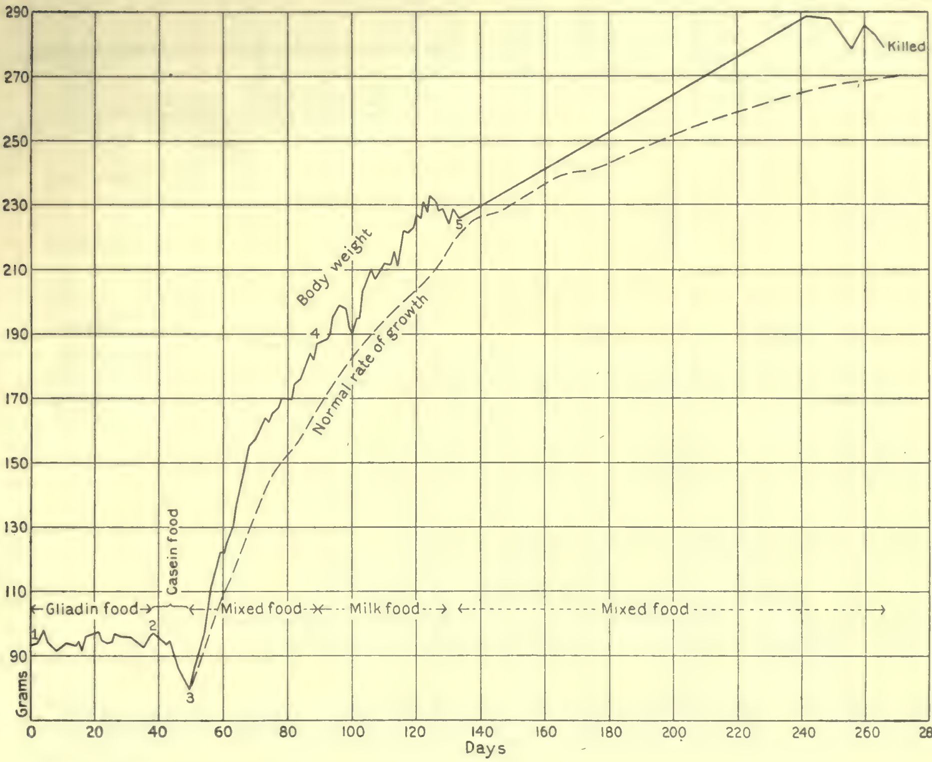


CHART XCVIII.

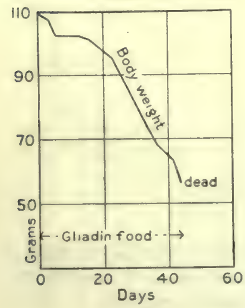


Chart XCVIII (rat 185, male) shows the failure of a rat to be maintained on a diet composed as shown herewith.

	p. ct.
Gliadin (from wheat)...	18.0
Starch.................	29.5
Sugar..................	15.0
Agar...................	5.0
Salt mixture I..........	2.5
Lard...................	30.0

CHART XCIX.

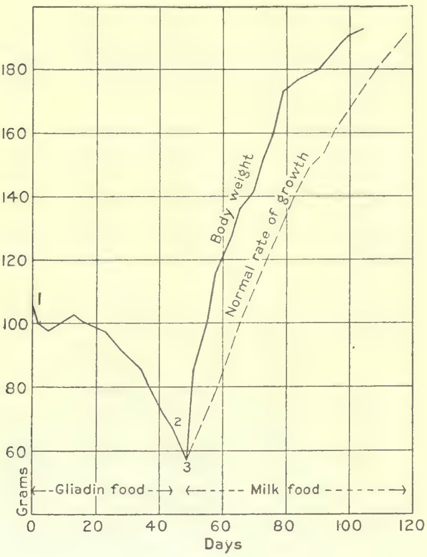
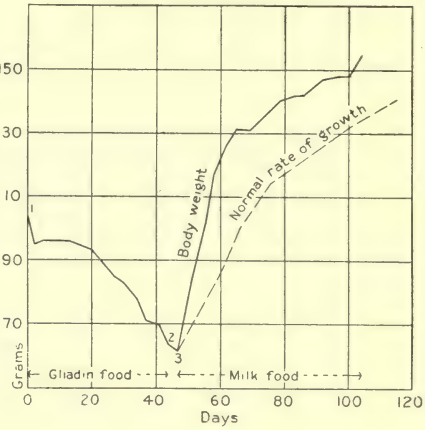


CHART C.



Charts XCIX (rat 186, male) and C (rat 188, female) show the failure of the rat to be maintained during periods 1 and 2 on diets mentioned below. The perfect resumption of growth when the diet consisted of milk-paste (period 3) illustrates that the "capacity to grow" normally is not visibly impaired by previous large loss of body-weight. The food consisted of—

Constituents.	Per. 1.	Per. 2.	Constituents.	Per. 3.
	p. ct.	*p. ct.*		*p. ct.*
Gliadin (from wheat)	18.0	0.0	Trumilk	60.0
Edestin	0.0	18.0	Starch	15.7
Starch	29.5	32.5	Salt mixture I	1.0
Sugar	15.0	17.0	Lard	23.3
Agar	5.0	5.0		
Salt mixture I	2.5	2.5		
Lard	30.0	25.0		

Chart CI (rat 147, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2), until fæces were added in period 3. The diet consisted of mixed food during period 1; for periods 2 and 3 it was as shown herewith.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

Chart CII (rat 142, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2). The addition of fæces to the diet in periods 3 and 4 checked the decline. During period 3 the fæces added were thoroughly sterilized and seemed to be less efficient than the unsterilized fæces in period 4, or in other similar experiments. The diet consisted of mixed food during period 1; for periods 2, 3, and 4 it was as shown herewith.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART CI.

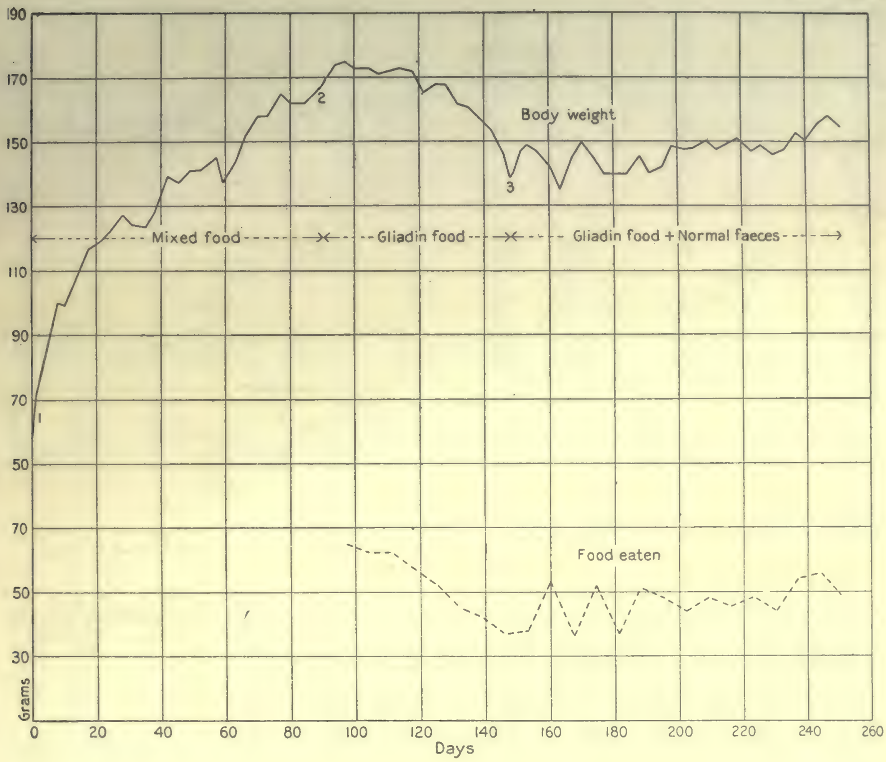


CHART CII.

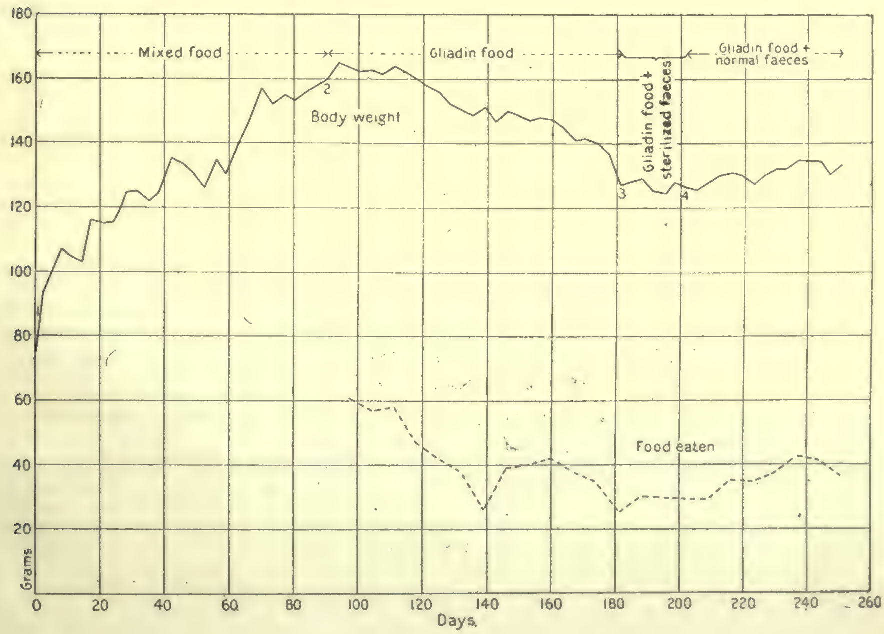


CHART CIII.

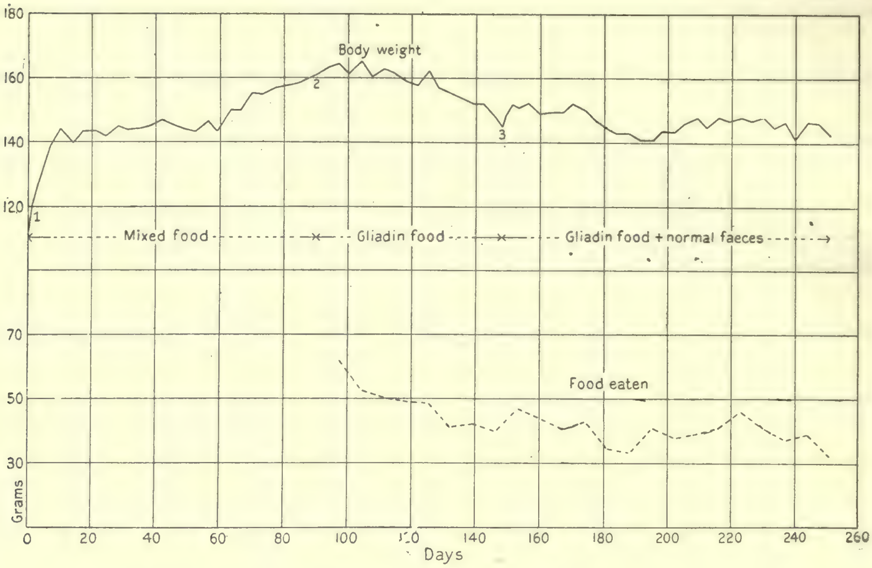


Chart CIII (rat 130, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2), until fæces were added in period 3. The diet consisted of mixed food during period 1; for periods 2 and 3 it was as shown.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART CIV.

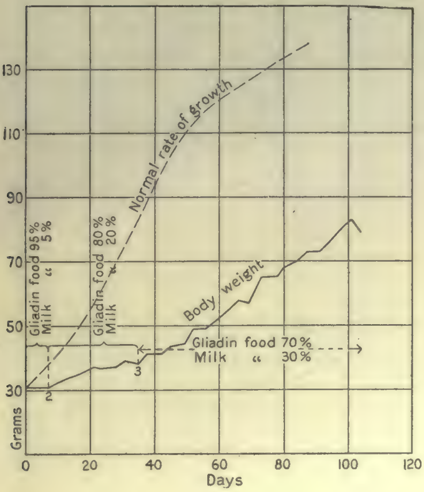


CHART CV.

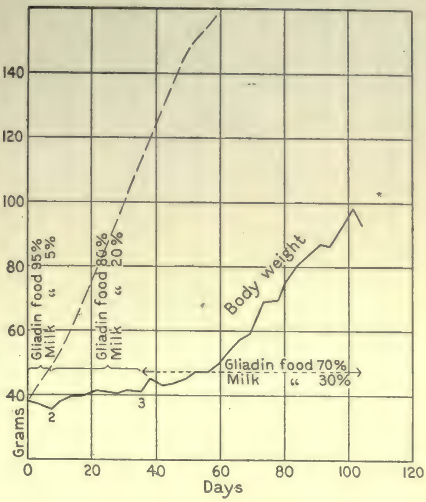


CHART CVI.

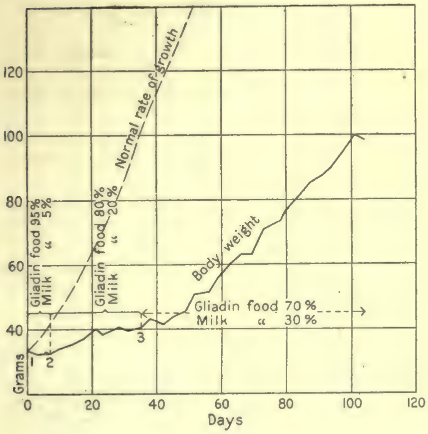
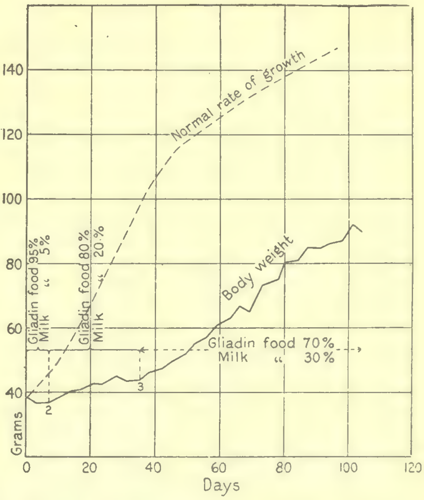


CHART CVII.



Charts CIV (rat 234, female), CV (rat 228, female), CVI (rat 235, male), and CVII (rat 227, male) show the effect of successively larger additions of milk-paste to gliadin food mixture which has been shown in other experiments to be inadequate for the purposes of growth. Note the more rapid growth as the content of milk is increased. The diet consisted of—

Constituents.	Per. 1.	Per. 2.	Per. 3.
	p. ct.	*p. ct.*	*p. ct.*
*Gliadin food.....	95	80	70
†Milk food.......	5	20	30

*Gliadin food: gliadin (from wheat) 18.0; starch, 29.5 to 32.5; sugar, 17.0; agar, 5.0: salt mixture I, 2.5; lard, 25 to 28.

†Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3.

Chart CVIII.

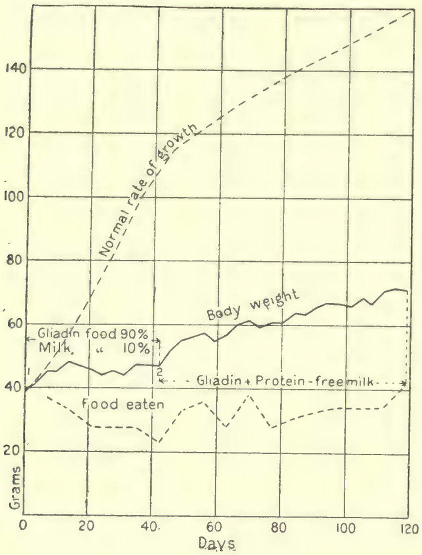


Chart CIX.

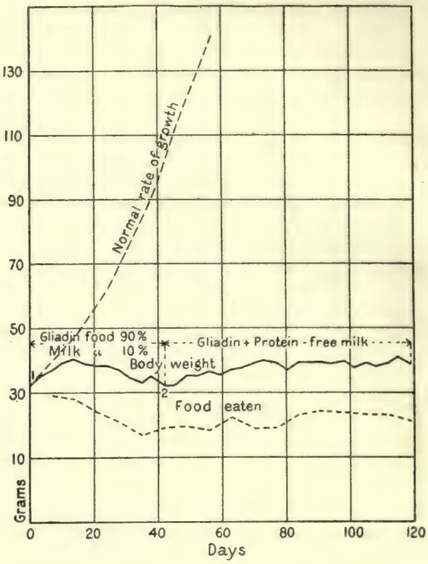


Chart CX.

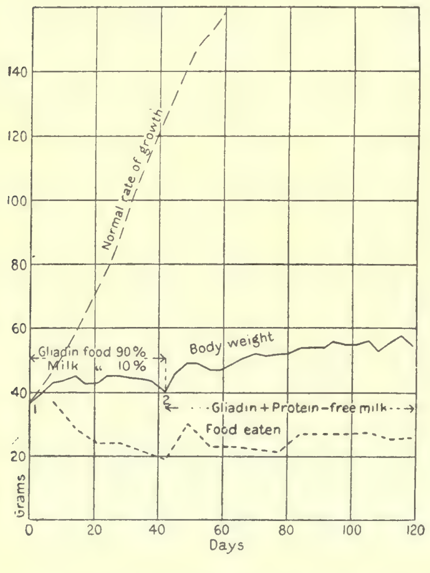
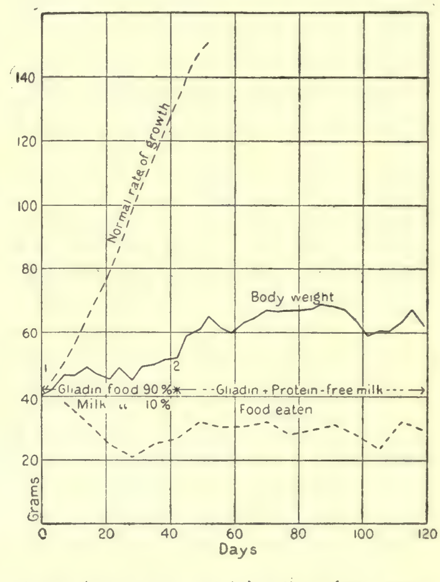


Chart CXI.



Charts CVIII (rat 214, female), CIX (rat 219, male), CX (rat 220, male), and CXI (rat 213, male) show the failure to induce more than slight growth when gliadin forms the sole protein of the dietary, even under conditions in which most other proteins have been found effective. That the failure to grow is not due to insufficient food intake is evident. The character of the diets is given in the table below.

Period 1:	*p. ct.*
Gliadin food: gliadin (from wheat), 18.0; starch, 32.5; sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0	90
Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3	10

Period 2:	*p. ct.*
Gliadin (from wheat)	18.0
Protein-free milk	28.2
Starch	20.8
Agar	5.0
Lard	28.0

CHART CXII.

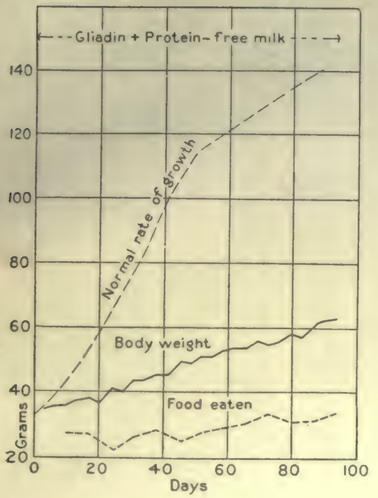


CHART CXIII.

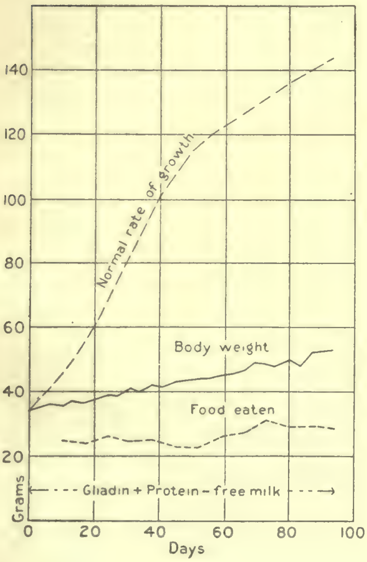
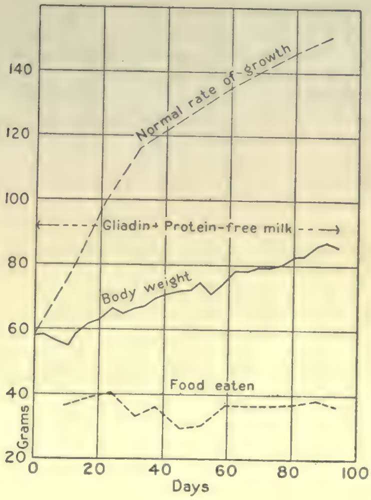


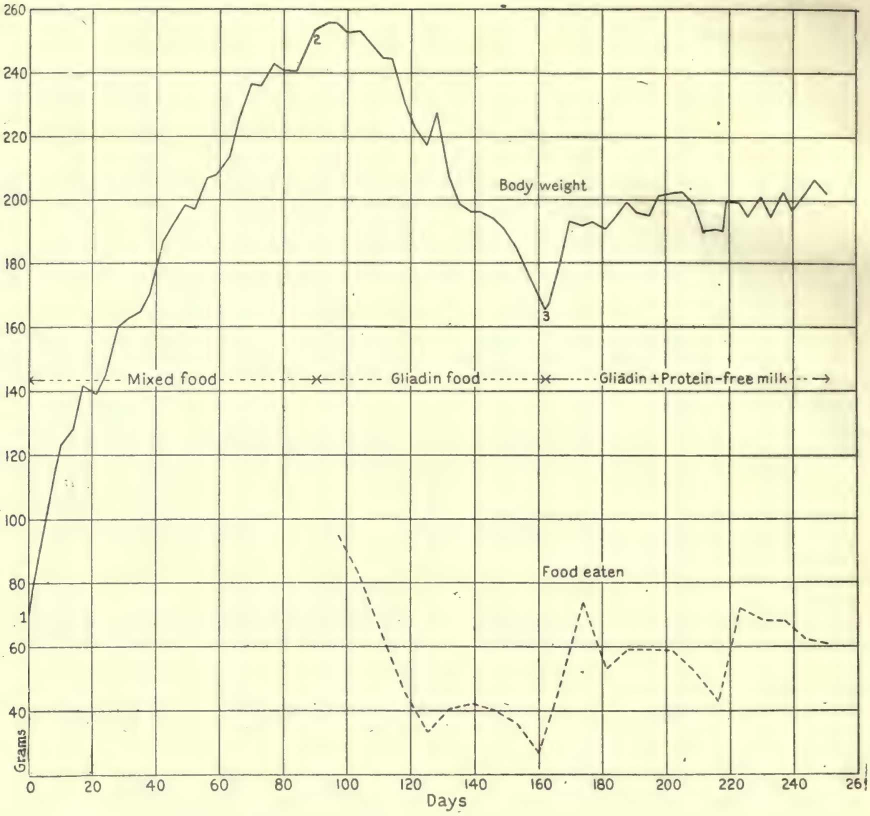
CHART CXIV.



Charts CXII (rat 249, female), CXIII (rat 240, female), and CXIV (rat 254, female) show the failure of the animals to grow normally on a diet containing protein-free milk and gliadin as the sole protein. It will be noted that these animals ate well and that the maintenance was better than with similar gliadin mixtures which contained no protein-free milk. The composition of the food was as follows:

	p. ct.
Gliadin (from wheat)	18.0
Protein-free milk	28.2
Starch	20.8
Agar	5.0
Lard	28.0

CHART CXV.



Charts CXV (rat 144, male) and CXVI (rat 134, female) show the failure of animals previously well nourished on a mixed diet (period 1), to be maintained on a diet in which gliadin formed the sole protein (period 2), until protein-free milk was added to the food (period 3). The food during period 1 was mixed. During periods 2 and 3 it was as shown herewith.

Constituents.	Per. 2.	Per. 3.
	p. ct.	*p. ct.*
Gliadin (from wheat)	18.0	18.0
Protein-free milk	0.0	28.2
Starch	29.5 to 34.5	20.8
Sugar	15.0 17.0	0.0
Agar	5.0	5.0
Salt mixture I	2.5	....
Lard	25.0 30.0	28.0

Chart CXVII (rat 129, female) shows the maintenance in period 2 on a diet containing protein-free milk and gliadin as the sole protein. Note that the animal did not decline like those fed on gliadin without protein-free milk. The preliminary period 1 on a mixed diet, during which the animal was twice pregnant, is introduced to show the excellent previous nutritive condition of the rat. The composition of the food for period 1 was mixed; for period 2 it was as shown herewith.

	p. ct.
Gliadin (from wheat)	18.0
Protein-free milk	28.2
Starch	20.8
Agar	5.0
Lard	28.0

CHART CXVI.

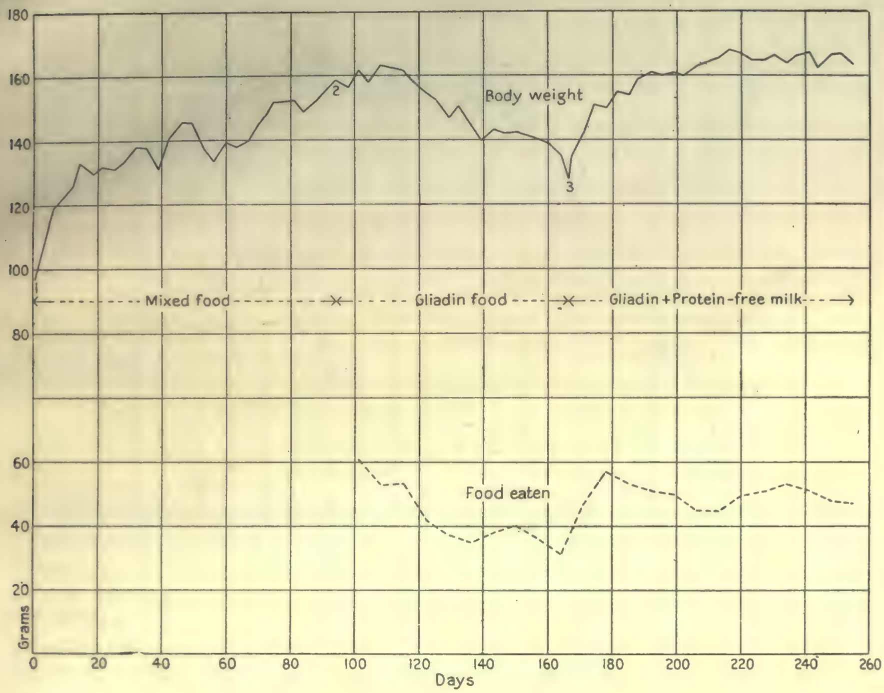


CHART CXVII.

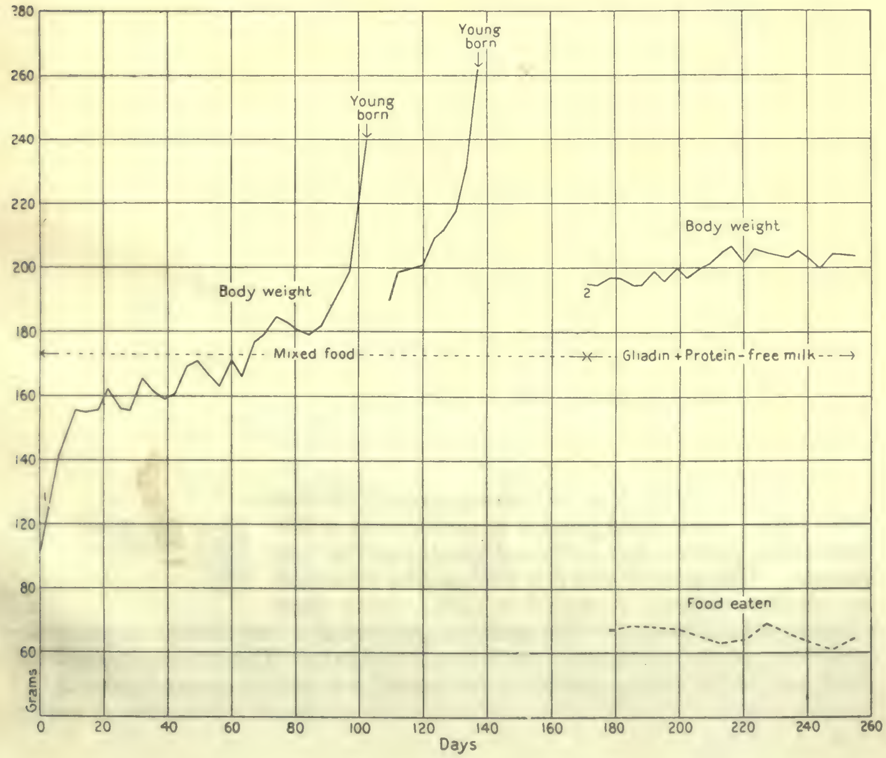


CHART CXVIII.

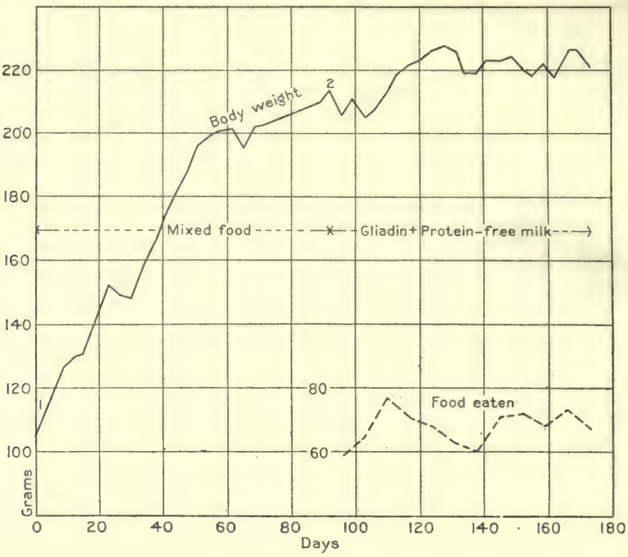
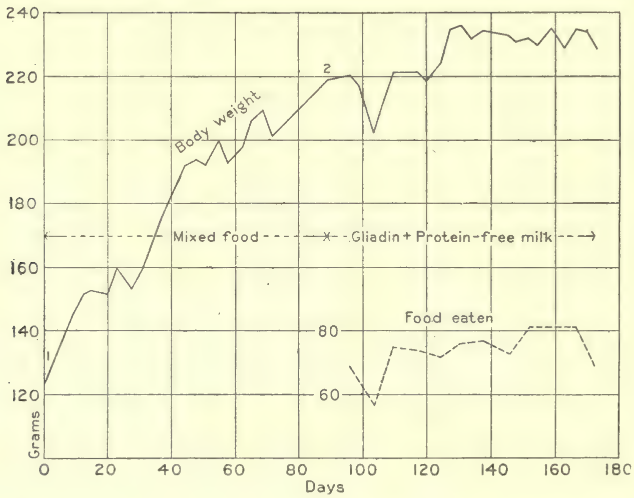


CHART CXIX.



	p. ct.
Gliadin (from wheat)...	18.0
Protein-free milk.......	28.2
Starch..................	20.8
Agar....................	5.0
Lard....................	28.0

Charts CXVIII (rat 167, male) and CXIX (rat 168, male) show maintenance in period 2 on a diet containing protein-free milk and gliadin as the sole protein. The animals did not decline like those fed on gliadin without protein-free milk. Note their abundant food intake. The preliminary period is introduced to show the excellent previous nutritive condition of the rats. The composition of the food was mixed during period 1; for period 2 it was as shown herewith.

CHART CXX.

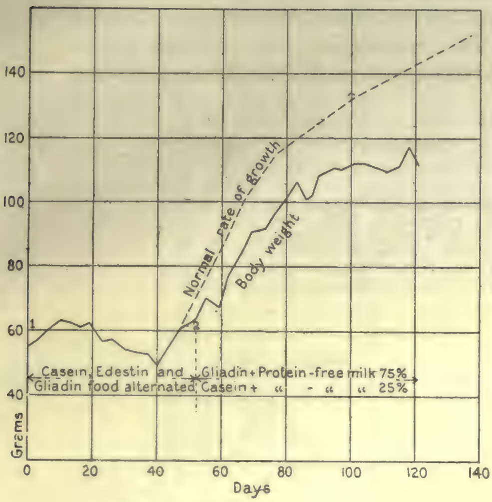
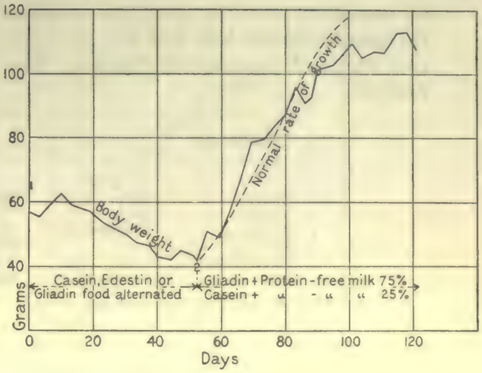


CHART CXXI.



Charts CXX (rat 208, female) and CXXI (rat 206, female) show, in period 1, failure to grow on the diet indicated below; and, in period 2, nearly normal growth on a diet containing protein-free milk in which one-quarter of the gliadin, previously found inadequate to induce growth, was replaced by casein. Note the small quantity of casein which suffices to promote growth instead of standstill. This emphasizes the different nutritive value of casein and gliadin. The diets consisted of—

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein or Edestin or Gliadin ...	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0)	75
Starch	32.5	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)	25
Sugar	17.0		
Agar	5.0		
Salt mixture I	2.5		
Lard	25.0		

CHART CXXII.

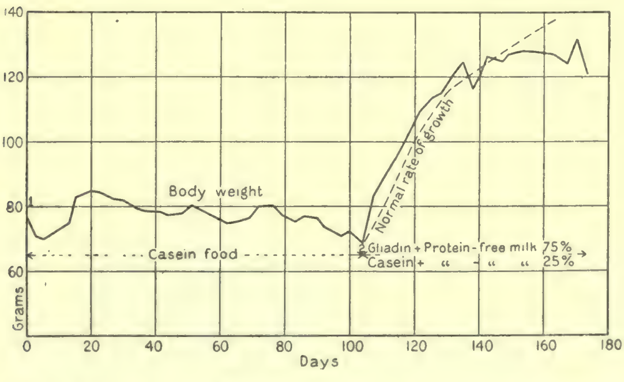


Chart CXXII (rat 179, female). Period 1 shows maintenance without growth on a diet containing salt mixture I (no protein-free milk) and casein as the sole protein. This should be contrasted with numerous similar experiments in which the inorganic constituents of the diet were present in the

form of protein-free milk. Period 2 shows the influence of the substitution by casein of one-fourth of the gliadin in a dietary repeatedly shown to suffice for maintenance but not for growth. This emphasizes the different nutritive value of casein and gliadin. The composition of the diets was as shown below.

Constituents.	Per. I.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein..........	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0).	75
Starch..........	32.5		
Sugar...........	17.0 to 20.0		
Agar............	5.0	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)............	25
Salt mixture I...	2.5		
Lard...........	22.0 to 25.0		

CHART CXXIII.

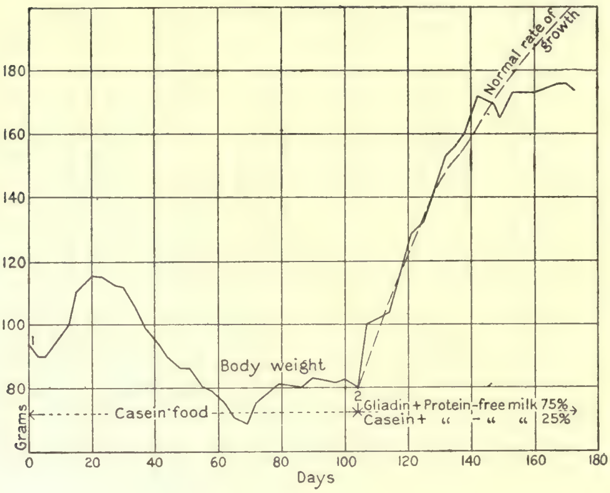


Chart CXXIII (rat 173, male). Period 1 shows imperfect maintenance without growth on a diet containing salt mixture I (no protein-free milk) and casein as the sole protein. This should be contrasted with numerous similar experiments in which the inorganic constituents of the diet were present in the form of protein-free milk. Period 2 shows the influence of the substitution by casein of one-fourth of the gliadin in a dietary repeatedly shown to suffice for maintenance but not for growth. This emphasizes the different nutritive value of casein and gliadin. The composition of the diets was—

Constituents.	Per. I.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein............	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0.).	75
Starch...........	32.5		
Sugar............	17.0 to 20.0		
Agar.............	5.0	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)..................	25
Salt mixture I....	2.5		
Lard.............	22.0 25.0		

CHART CXXIV.

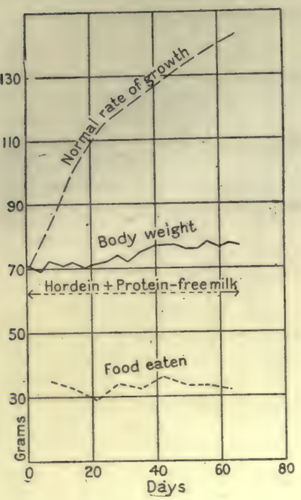
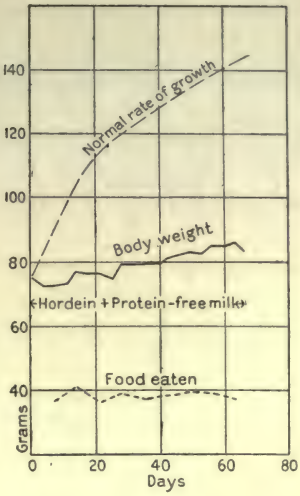


CHART CXXV.



Charts CXXIV (rat 256, female) and CXXV (rat 255, female) show maintenance without growth of medium-sized rats on a diet of protein-free milk and hordein, from barley, as the sole protein. Note the undiminished appetite during course of experiment. Precisely similar mixtures containing other single proteins have sufficed to induce growth. This experiment demonstrates the different nutritive value of hordein and most other proteins and its resemblance in this respect to the chemically similar protein gliadin. This is a marked instance of the relation of the chemical constitution of the protein to nutrition. The composition of the food was as shown herewith.

	p. ct.	
Hordein	18.0	....
Protein-free milk	28.2	....
Starch	16.8 to	18.8
Agar	5.0	....
Lard	30.0	32.0

Chart CXXVI.

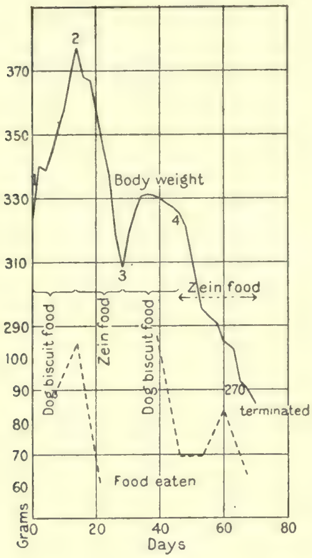
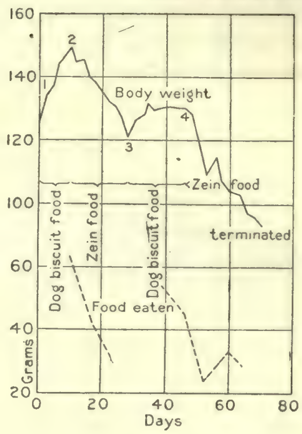


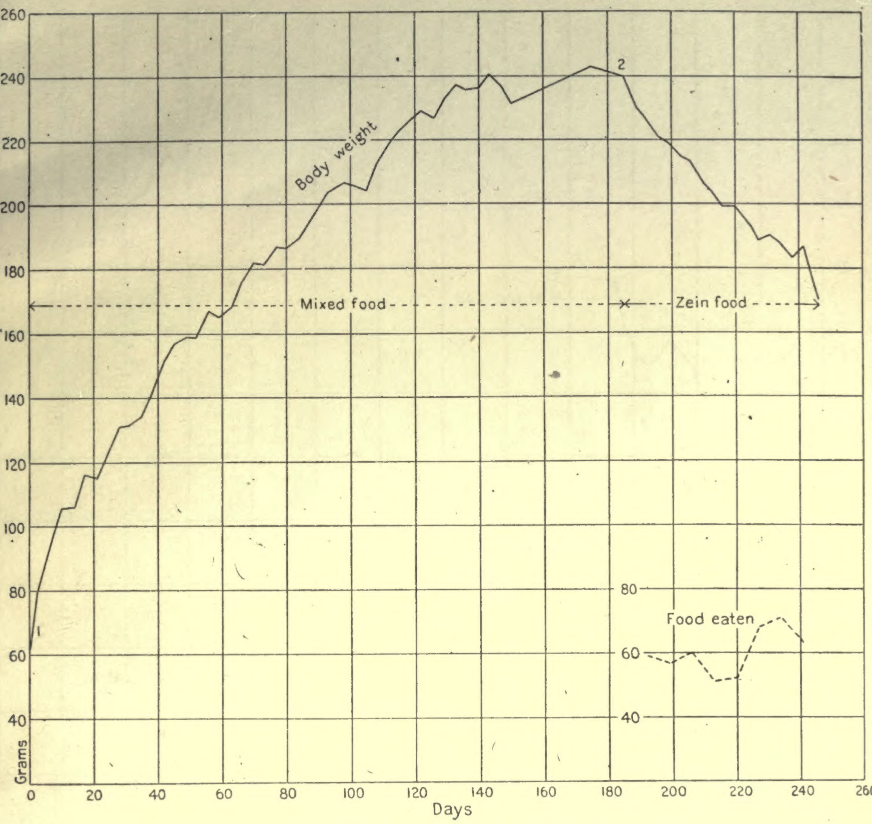
Chart CXXVII.



Charts CXXVI (rat XI) and CXXVII (rat XIV) show the failure of well-nourished animals (see period 1) to be maintained on a diet in which zein formed the sole protein. The diet consisted of—

Constituents.	Periods 1 and 3.	Constituents.	Per. 2.	Per. 4.
	p. ct.		*p. ct.*	*p. ct.*
Dog biscuit......	58.33	Zein..........	16.89	10.77
Lard............	41.66	Starch.......	10.14	23.70
		Sugar........	8.78	21.54
		Salt mixture..	3.38	2.15
		Agar.........	10.14	5.17
		Lard.........	50.67	36.63

CHART CXXVIII.



Period 2.

	p. ct.
Zein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

Chart CXXVIII (rat 146, male) shows the failure of a well-nourished rat (see period 1) to be maintained on a diet containing protein-free milk and zein as the sole protein. It should be noted that precisely similar mixtures in which zein was replaced by any of the other proteins studied, sufficed either to induce growth or at least to maintain body-weight for an equally long period. Attention is directed to the continued fall in weight despite the large food intake. The composition of the food was mixed for period 1; for period 2 it was as shown herewith.

CHART CXXIX.

Chart CXXIX (rat 157, male) shows the failure of a well nourished rat (see period 1), to be maintained on a diet containing protein-free milk and zein as the sole protein. It should be noted that precisely similar mixtures in which zein was replaced by any of the other proteins studied, sufficed either to induce growth or at least to maintain body-weight for an equally long period. Attention is directed to the continued fall in weight despite the large food intake. The composition of the food was mixed for period 1; for period 2 it was as shown herewith.

Period 2.	*p. ct.*
Zein..............	18.0
Protein-free milk.	28.2
Starch...........	23.8
Agar.............	5.0
Lard.............	25.0

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